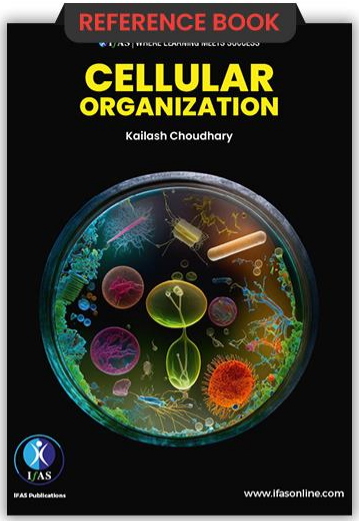
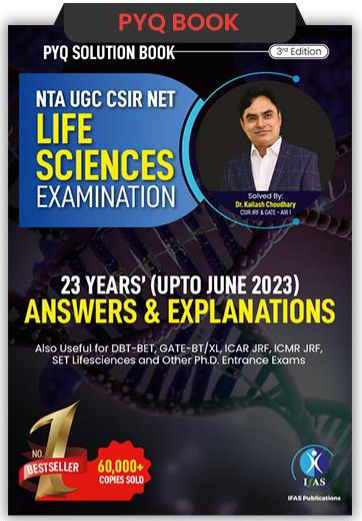
Don't wait for the opportunity. Create it.

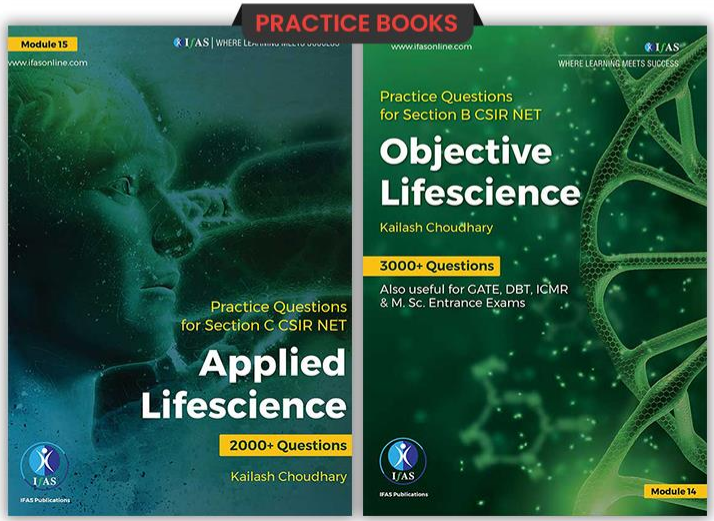
CSIR NET – Life Science

Unit 2: Cellular Organization

L7 Cell Organelles, Secretory Protein







IfAS

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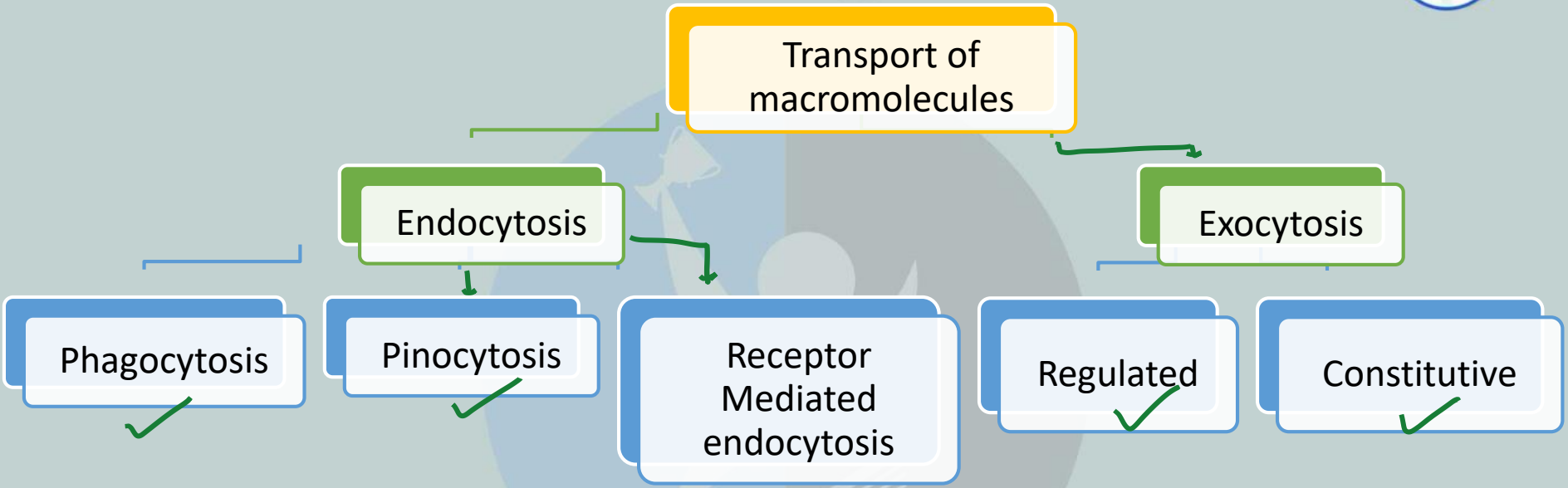
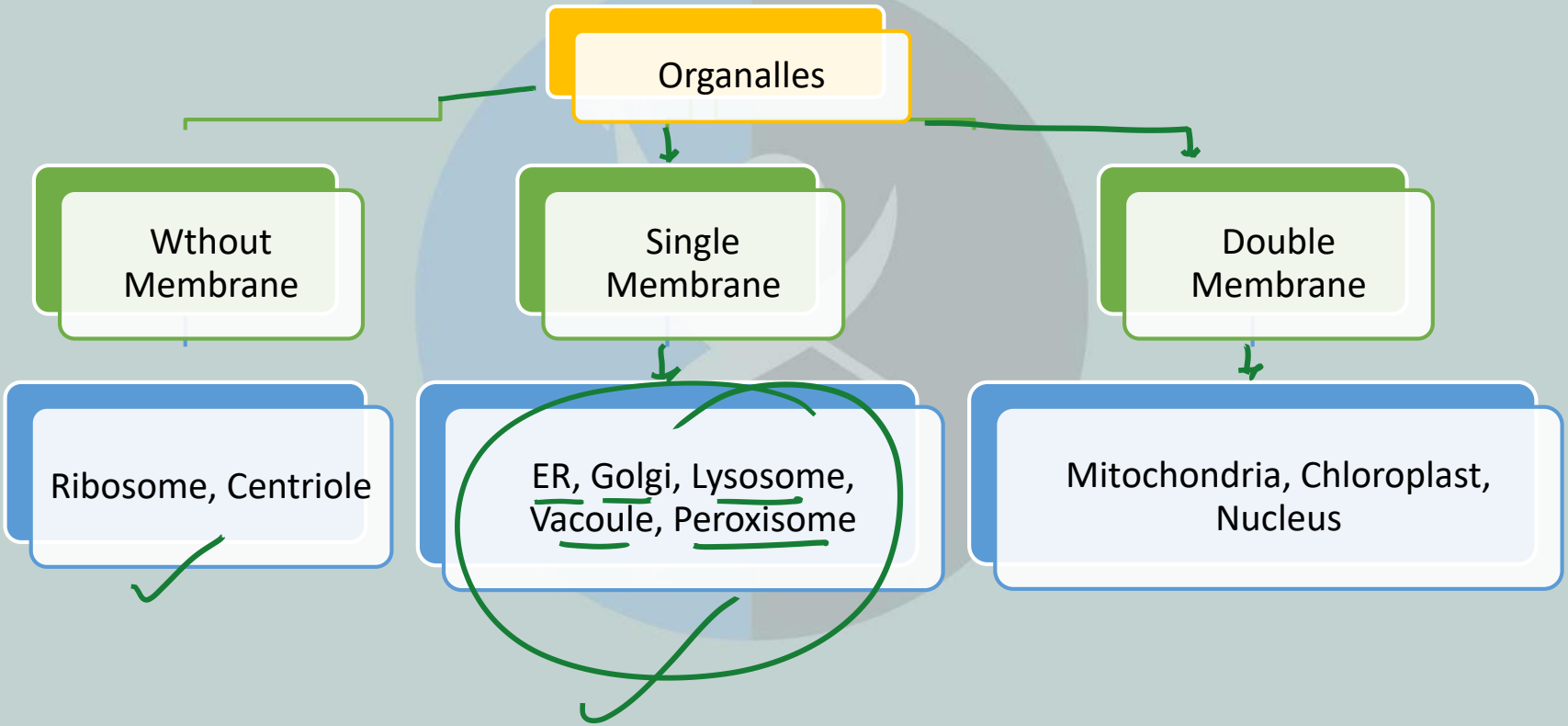
Points to be covered in this Lecture

- Cytosolic and secretory protein
- Signal peptide and Targeting to RER
- Membrane proteins signals
- Unfolding protein response
- ER, Golgi, Lysosome

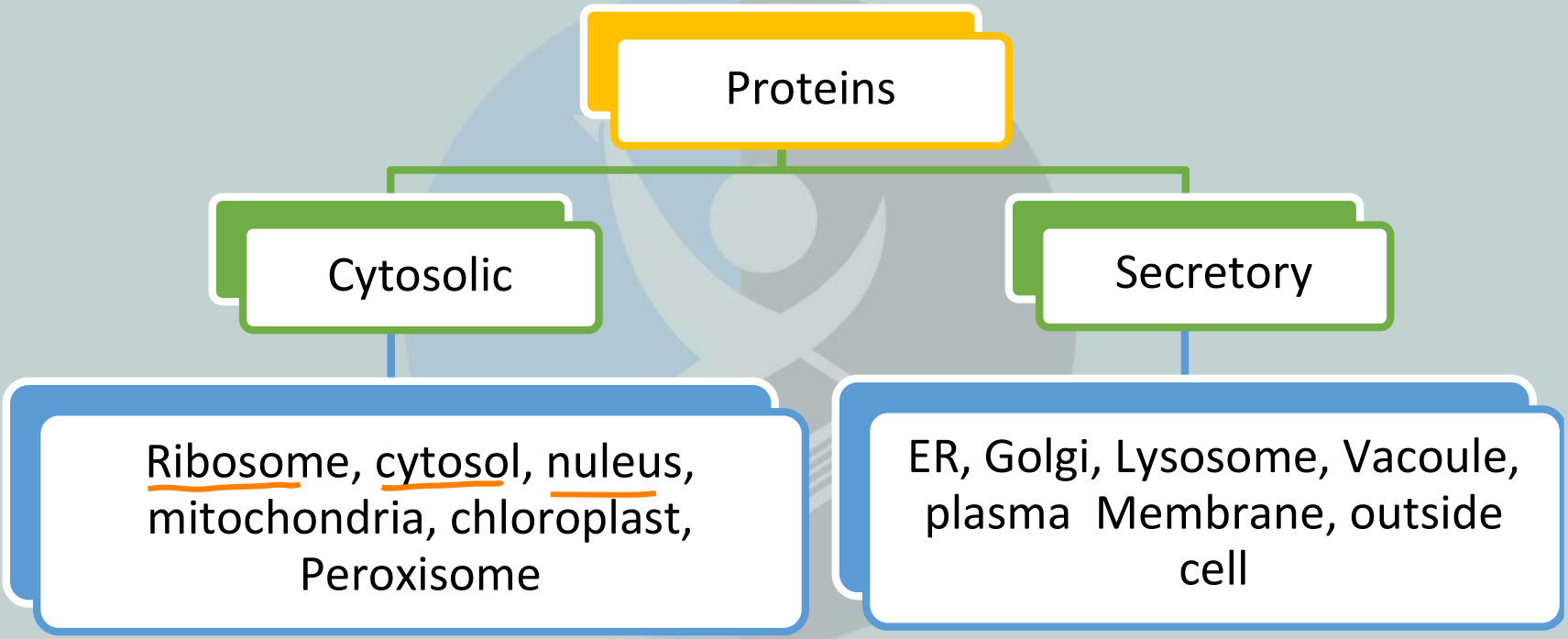


- **Phagocytosis:** Cell eating, where cells engulf and internalize solid particles such as bacteria and viruses. Example: Macrophage
- **Pinocytosis:** Cell drinking, cells internalize small dissolved particles, such as ions, sugars, and amino acids, from their extracellular environment.
- **Receptor mediated endocytosis:** cells internalize specific molecules that bind to receptor proteins on their cell surface. Example: Uptake of LDL particles.

Organelles of Eukaryotic Cells

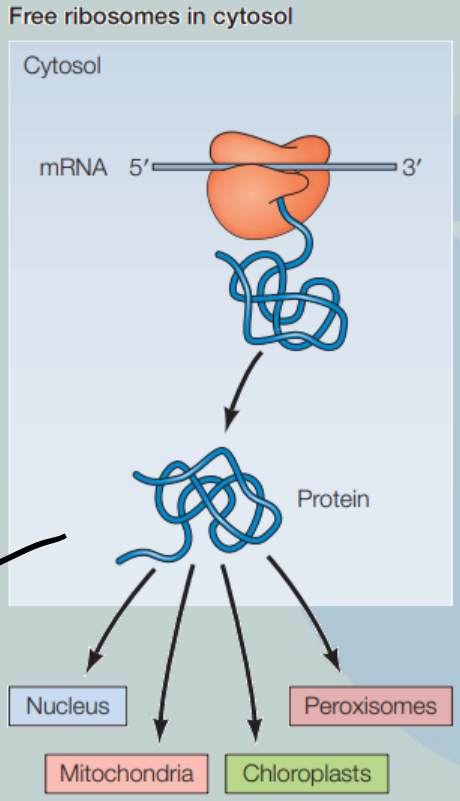


Cytosolic and Secretory Protein

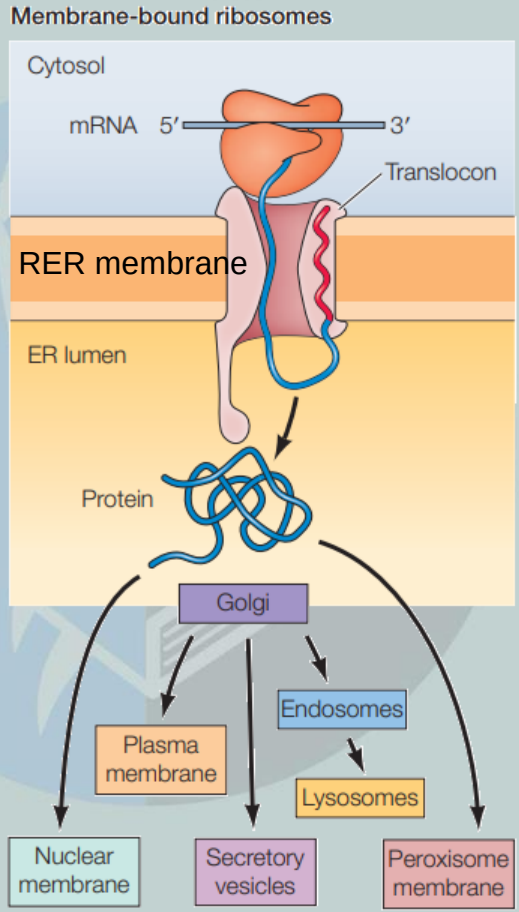


Cytosolic protein

Synthesis starts and complete on free ribosome



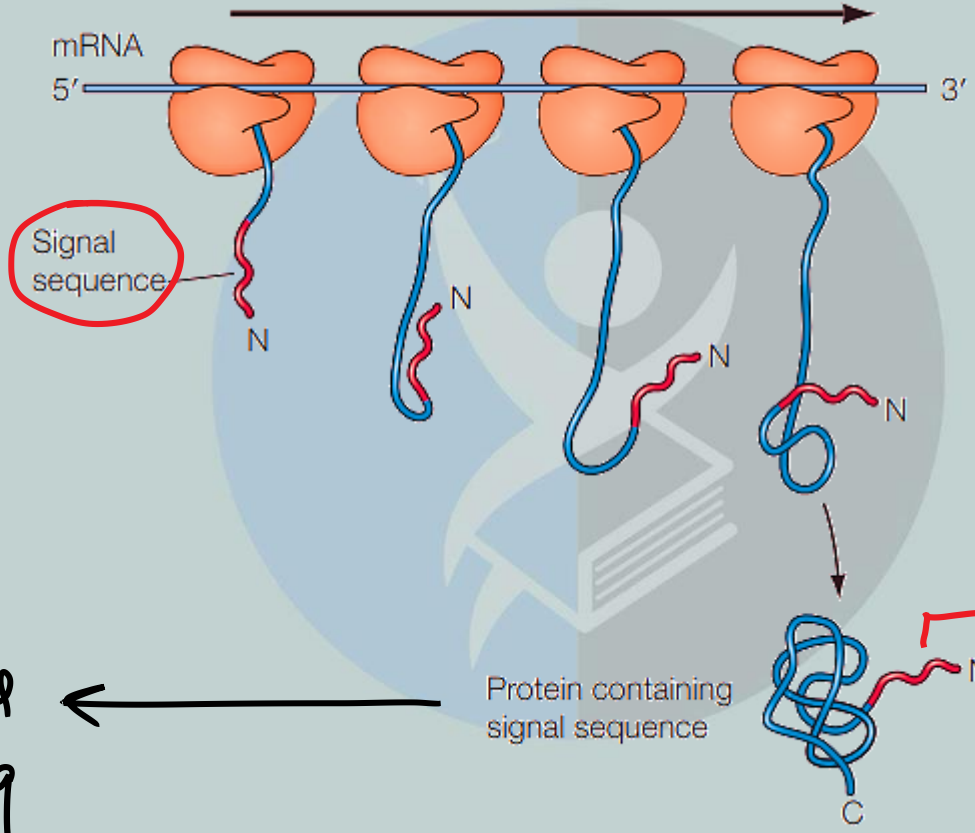
Cytosol



Secretory protein

- Synthesis starts on free ribosome
- But completed on RER membrane

Translation on free ribosomes

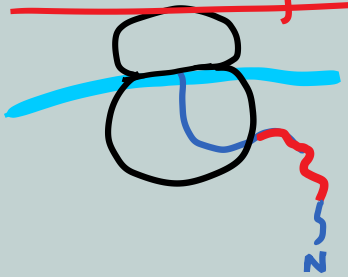


Cytosolic Protein

Post Translational Targetting

Helps in Protein Targetting

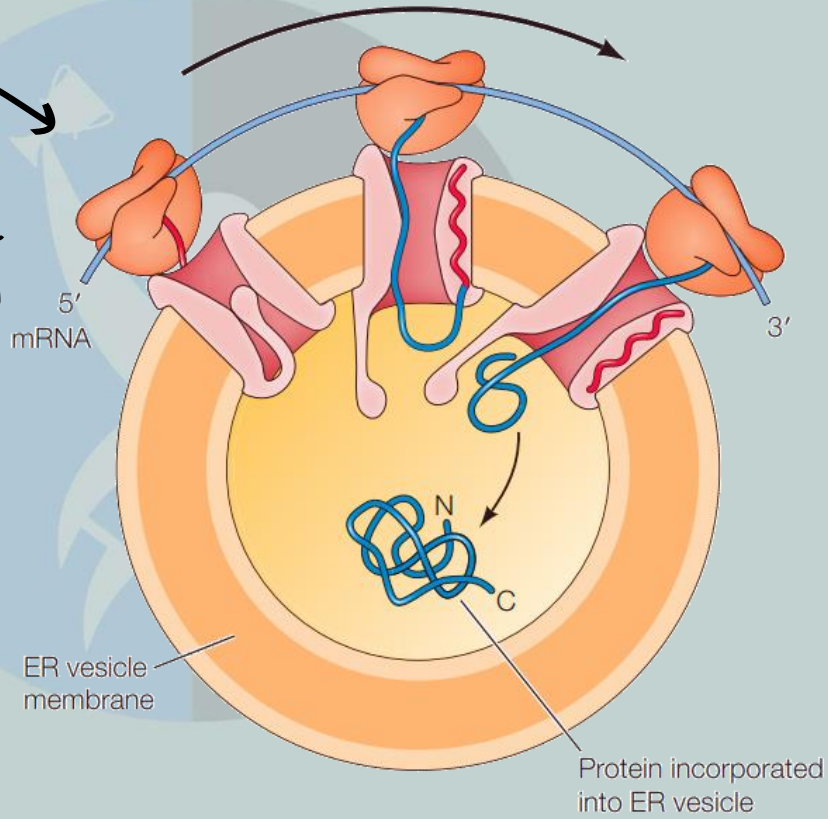
Secretory Protein



Free Ribosome

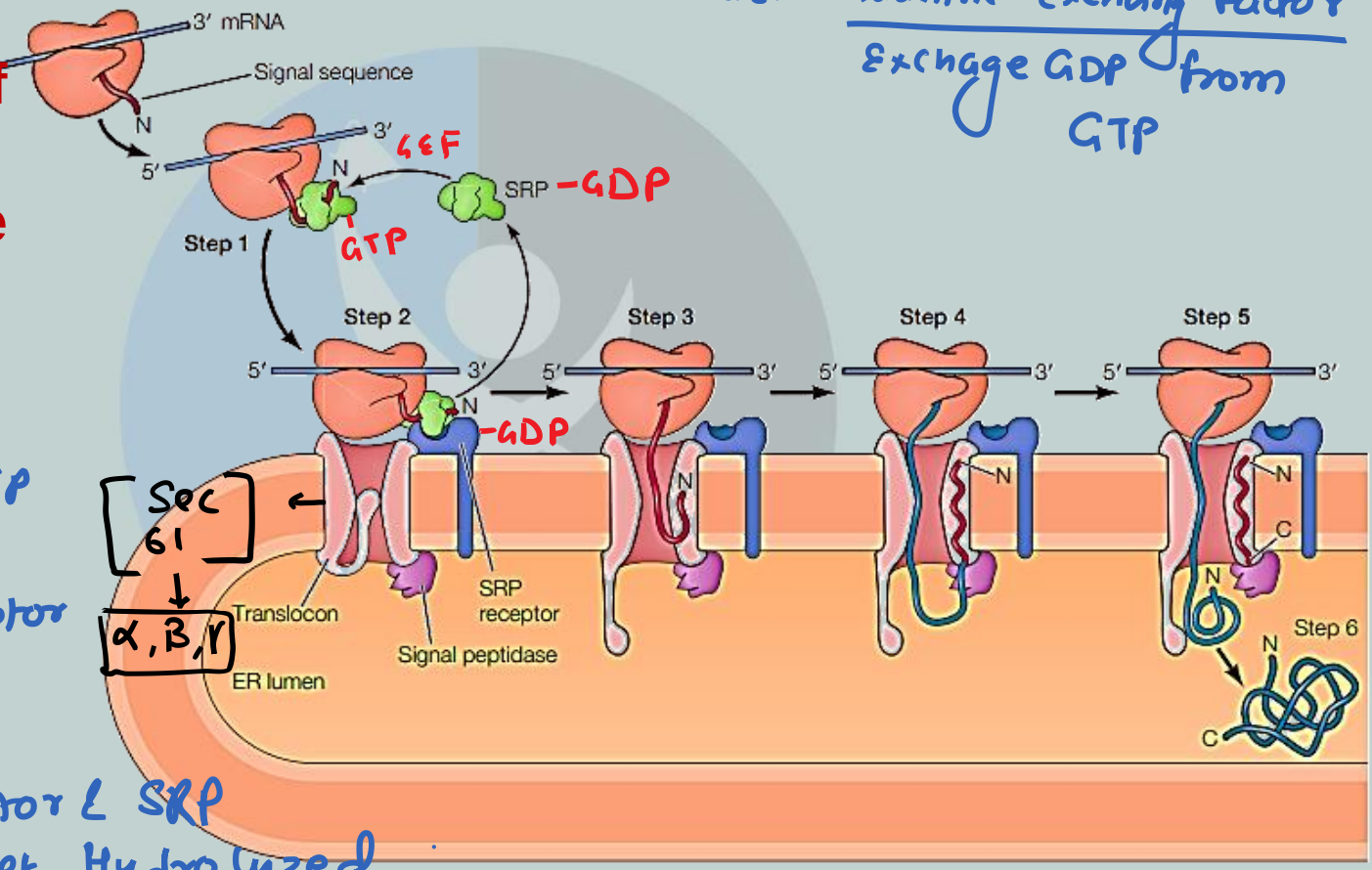
Co-Trans-lational Targetting

Translation with ER vesicles present



Cotranslational targeting of secretory proteins to the ER

GEF: Guanine Exchang Factor
Exchgge GDP from GTP



SRP-GTP

SRP-Receptor-GTP

↓

SRP + SRP Receptor

GTP GTP

↓

GTP of Both Receptor & SRP Get Hydrolyzed

Hydrophobic signal s Recognize by Signal Recognition Particle [SRP] (SRP)

(16-30 Hydrophobic a.a)

Ribonucleoprotein - 300 nt –RNA
+
54 KD protein SRP

→ Ribonucleoprotein
↓
SRP

SRP interacts with Ribosome → Translation Pause

SRP interacts with –

1. signal peptide
2. Large Subunit of Ribosome
3. SRP Receptor present on RER membrane

Step 1: As the signal sequence emerges from the ribosome, it is recognized and bound by the signal recognition particle (SRP).

Step 2: The SRP escorts the complex to the ER membrane where it binds to the SRP receptor.

Step 3: The SRP is released, the ribosome binds to the translocon, and insertion of the signal sequence opens the translocon.

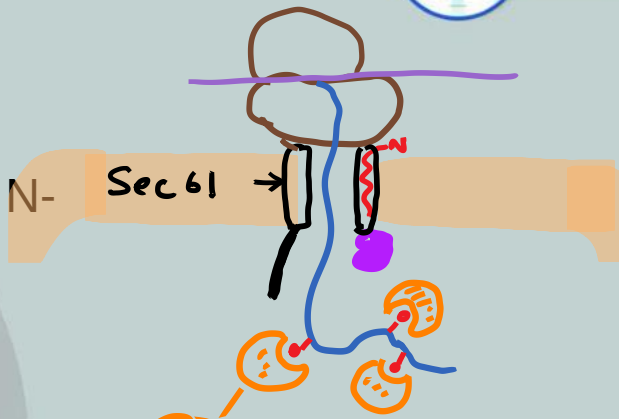
Step 4: Translation resumes and the signal sequence is cleaved by signal peptidase.

Step 5: Continued translation drives translocation of the growing polypeptide chain across the membrane. Step

6: The completed polypeptide chain is released within the ER lumen.

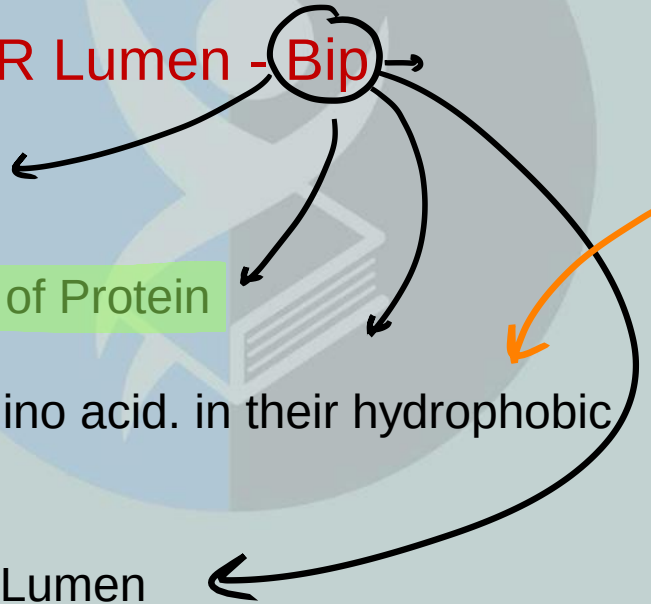
Signal peptidase

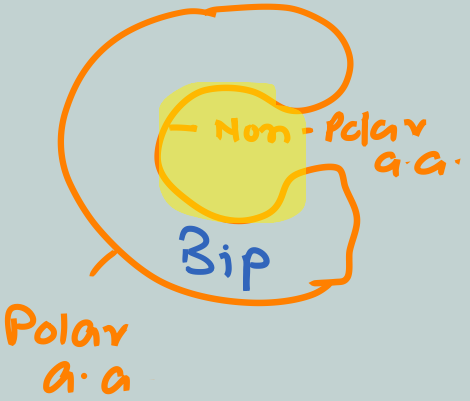
- RER Lumen - Cleave signal Peptide & Generate New N-Terminus

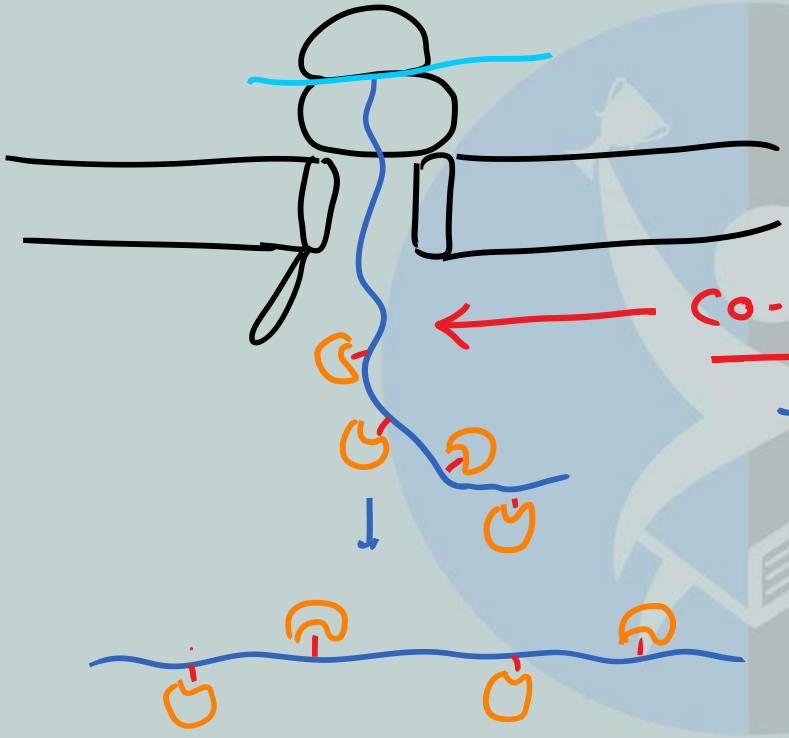


Chaperon present in RER Lumen - Bip

- Belongs to hsp70 family
- Prevent pre-mature folding of Protein
- Hiding the Hydrophobic amino acid. in their hydrophobic pocket
- Assist Protein folding RER Lumen







Co-Translational modification

→ modification on Protein During Translation.

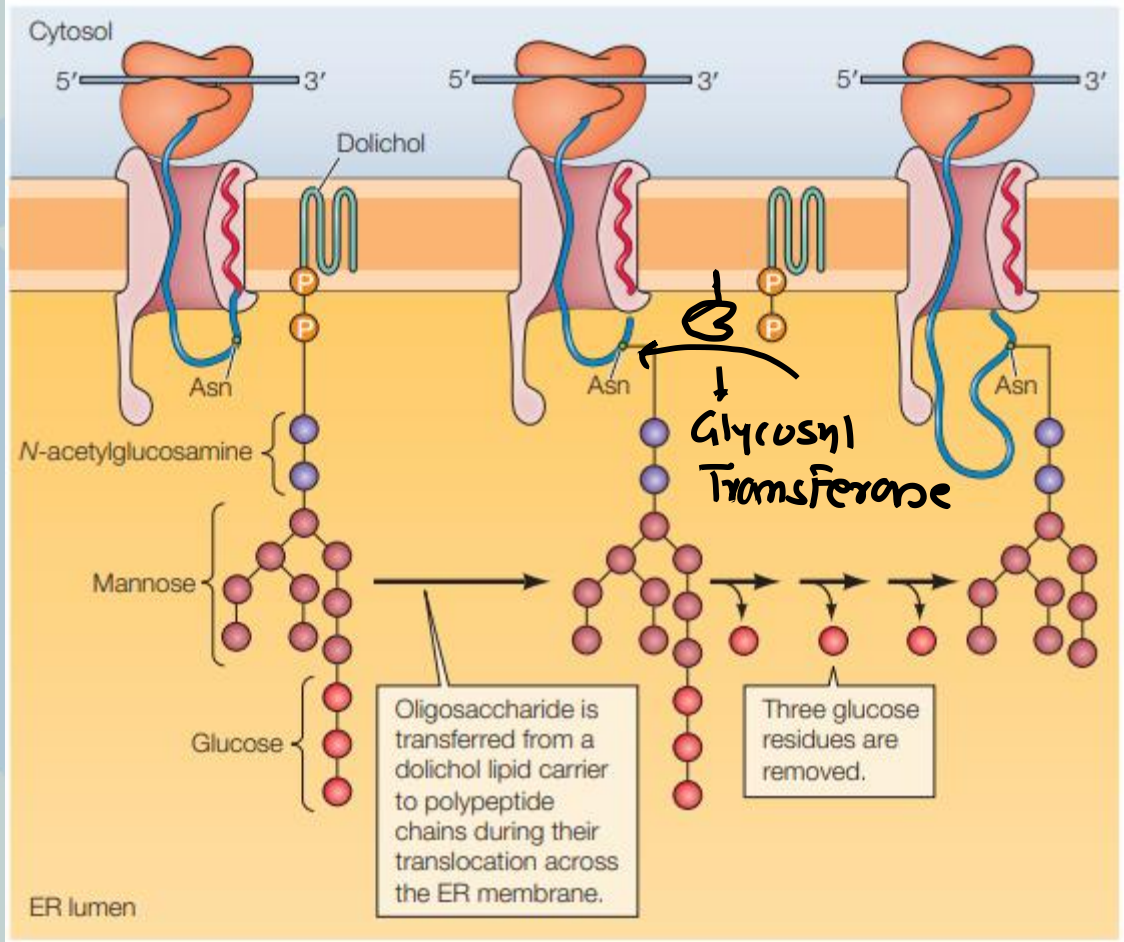
e.g. N-linked Glycosylation.

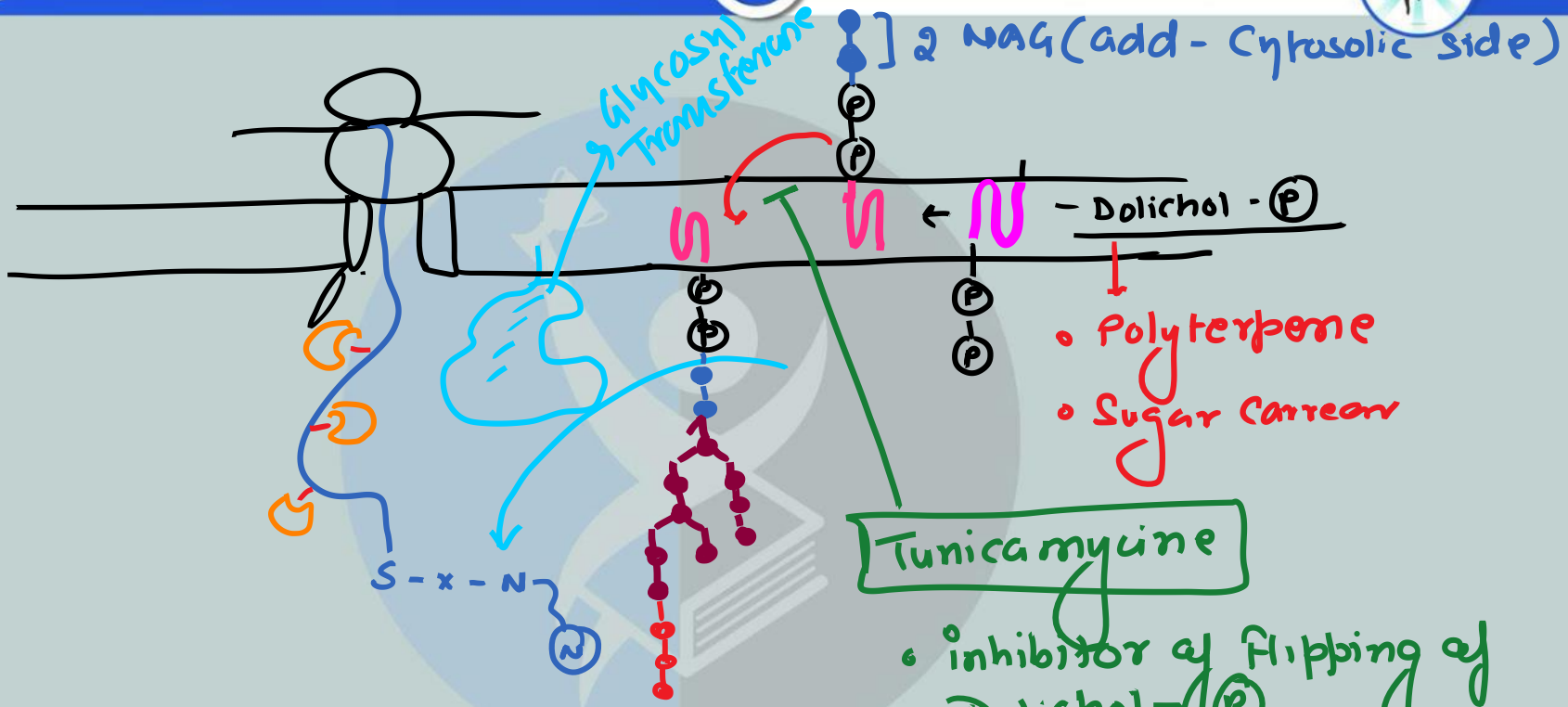
N-linked glycosylation

- Important for Protein Folding
- Glycosylation provide stability to the protein

Proteins are glycosylated within the ER by the addition of a 14-sugar oligosaccharide to an acceptor asparargine (Asn) residue.

Three glucose residues are removed while the protein is still within the ER





- Polyterpene
- Sugar Carrear

- inhibitor of flipping of Dolichol - P

↓

N-linked Glycosylation inhibited

The oligosaccharide is synthesized on a lipid carrier anchored in the ER membrane.

→ Dolichol.

It is then transferred as a unit to acceptor asparagine residues in the consensus sequence Asn-X-Ser/Thr

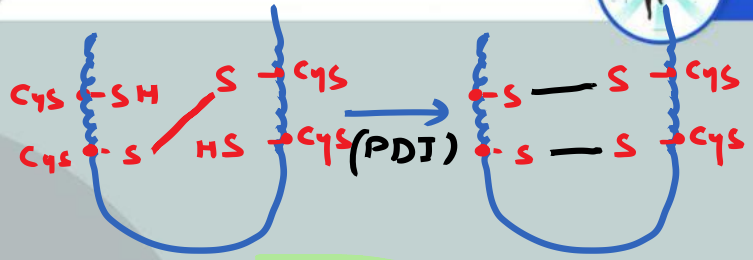
Glycosylation helps to prevent protein aggregation in the ER and provides signals that promote protein folding and subsequent sorting in the secretory pathway.

Modifications in RER Lumen

1. N-linked Glycosylation ✓
2. Di-Sulphide Bond formation
   By Protein-Di Sulphide isomarase
3. Prolyl cis Trans-isomarase
4. Quality Control of Folded protein
   - ER-Associated degradation
   - Unfolded protein Response

] unfolded/misfolded protein

Protein Di-Sulphide isomerase- [PDI]



- Helps in Di-sulphide Bond formation-
- Rearrangement of Di-sulphide Bond
- Oxidative Environment present in RER Lumen → Di-sulphide Bond can formed

- Cytosolic environment – di sulphide Bond can not form in cytosolic protein (Reducing Env.)

- Except - Keratinocytes Some how maintain oxidative environment in cytosol

- Kiratin is cytosolic protein but contain s-s Bond

Prolyl cis - trans isomarase

Pro-Pro (cis ⇌ trans) → To fold the protein

Quality Control of Folded Protein

→ Properly folded Proteins. leaves RER Lumen and enters in Golgi

Chaparons(Bip) - Unflolding

misfolded protein → Transport to the cytosol (ER mediated degradation)

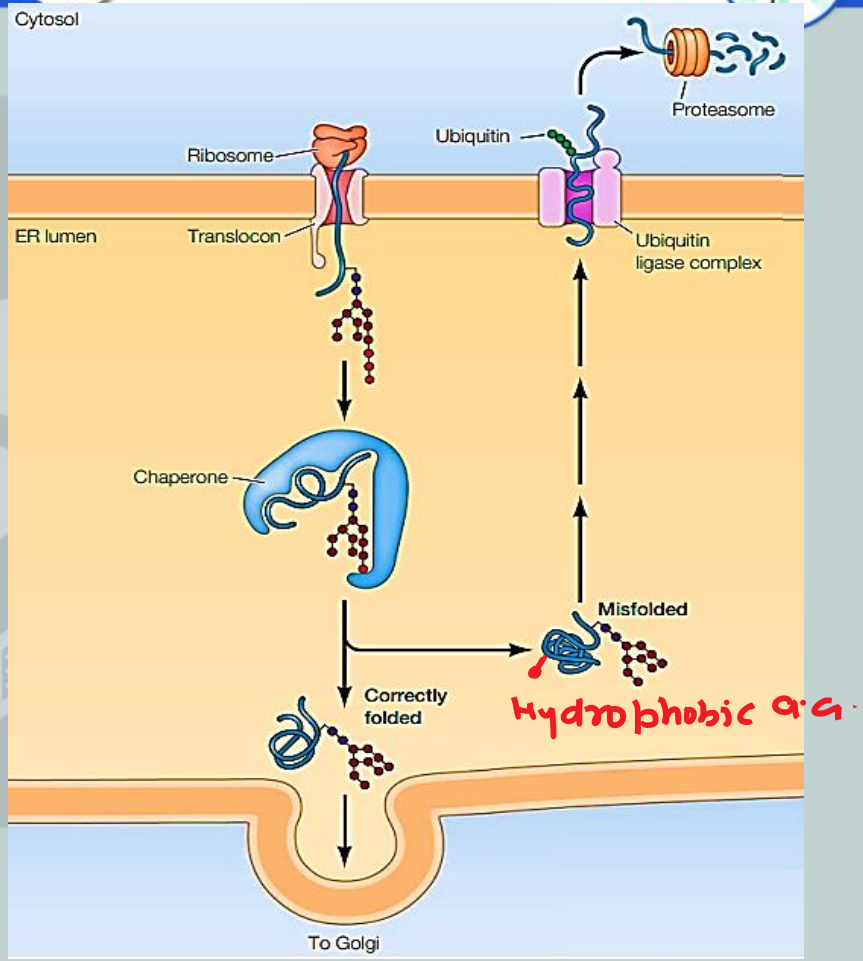
.Refold→ Correct Folding -- Packed into vesicle ----send into vesicle → Golgi

- Cal reticulin
- Calnexiin
- Glucosidase – I, II
- Mannosidase
- UDP Glycosyl Transferase

→ Enzymes involved in Quality control of Folded protein.

Glycoprotein folding

- As the glycoprotein exits the translocon, chaperones bind and assist in folding.
- If the protein is correctly folded it proceeds to exit the ER. → Golgi
- However, if too many hydrophobic regions are exposed, indicating improper folding, the protein is targeted back to the cytosol through a ubiquitin ligase complex in the ER membrane.
- The protein is ubiquitylated at the cytosolic side of this complex and degraded in the proteasome



If there is mutation in protein –(single copy mutation of protein)

Correct folding does not occurs

- ER Associated Degradation for mis-folded protein
- If RNA is mutated – unfolded protein Response (UPR)

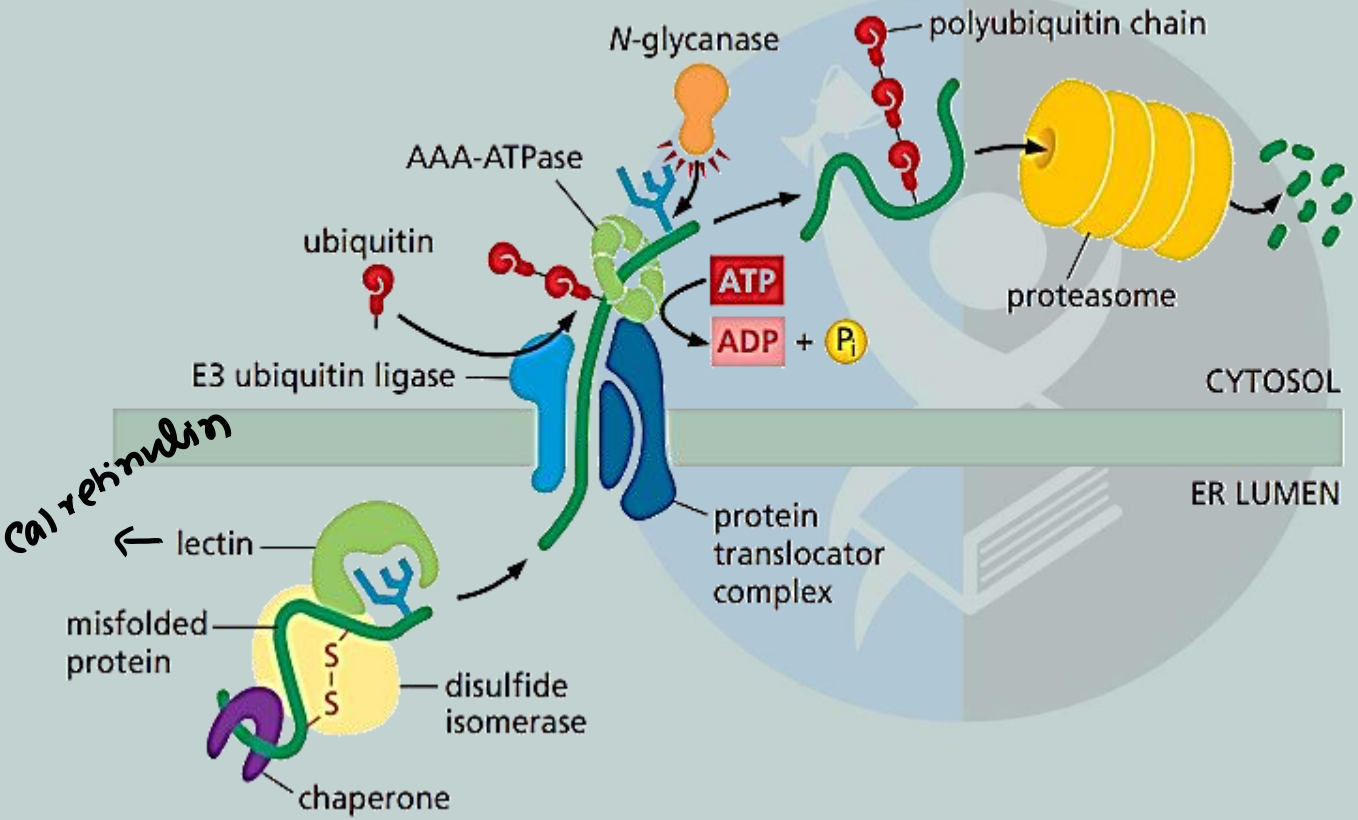
UPR

- Mutated RNA Degradation
- global translational inhibition (PER-K add P on eIF2 ⊣ Translation

- Chaperon expression

The export and degradation of misfolded ER proteins.

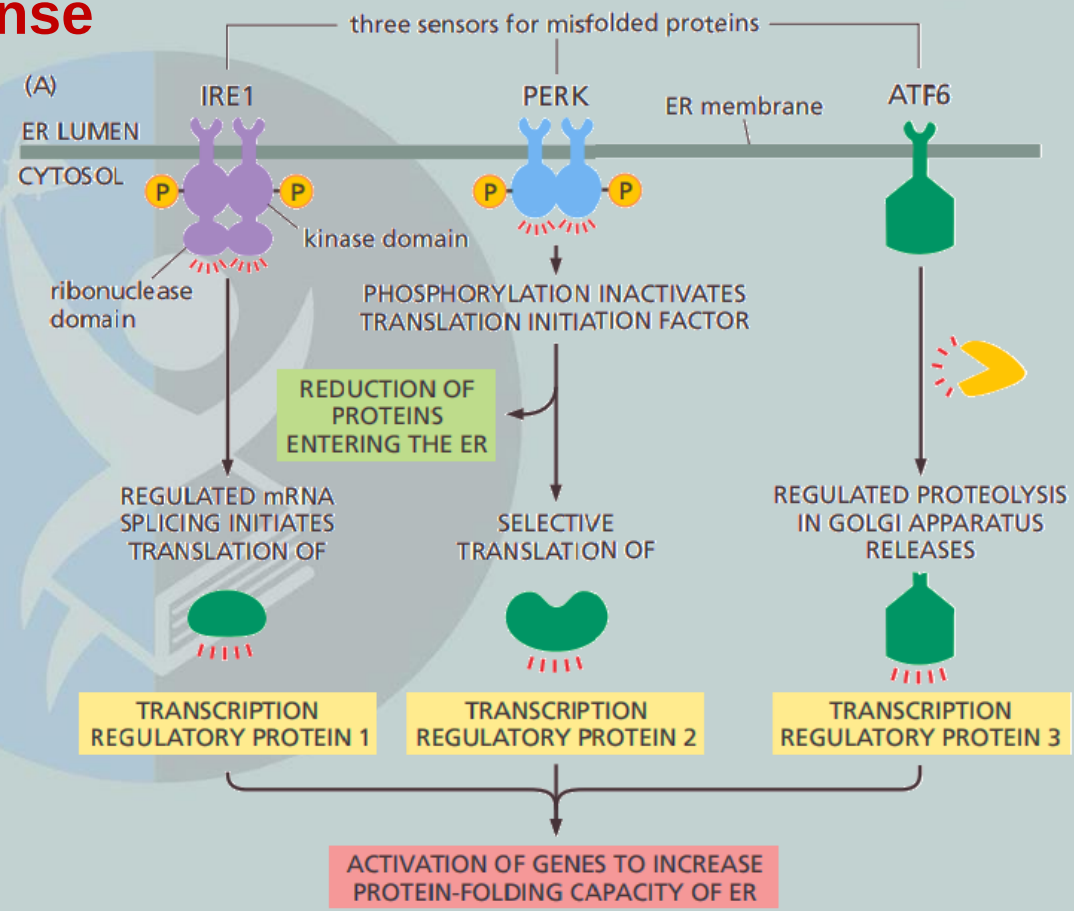
ER Associated Degradation.



- Misfolded soluble proteins in the ER lumen are recognized and targeted to a translocator complex in the ER membrane.
- They first interact in the ER lumen with chaperones, disulfide isomerases, and lectins.
- They are then exported into the cytosol through the translocator.
- In the cytosol, they are ubiquitylated, deglycosylated, and degraded in proteasomes.
- Misfolded membrane proteins follow a similar pathway but

The unfolded protein response

(A) By three parallel intracellular signaling pathways, the accumulation of misfolded proteins in the ER lumen signals to the nucleus to activate the transcription of genes that encode proteins that help the cell cope with misfolded proteins in the ER.



Synthesis of membrane protein

→ ER, Golgi, P.M, Nuclear, Peroxysome Lysosome

→ On the basis of Topology, Transmembrane Region

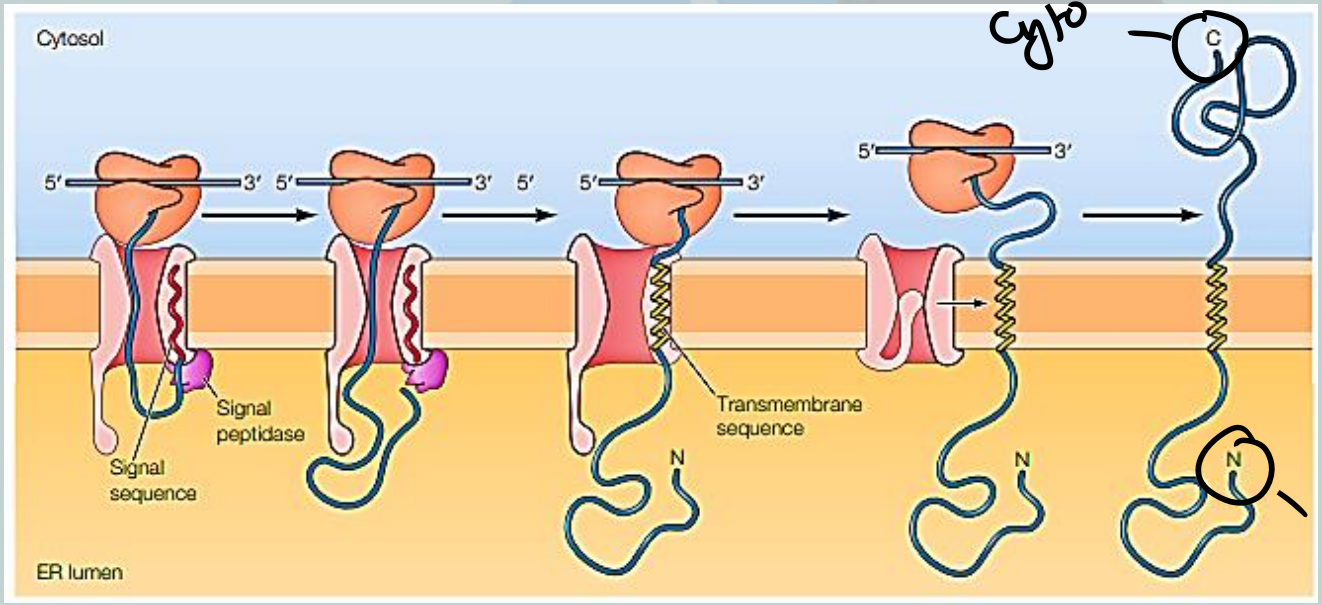
↓

- Transmembrane protein are different types

4 Types → Type I, Type II, Type III, Type IV

Insertion of a membrane protein with a cleavable signal sequence → Type I → e.g. Glycophorin
• LDL Receptor

Single Spaning Region → (STS)



Cyto

STS

– Stop Transfer Signal

Exoplasm.

- Insertion of a membrane protein with a cleavable signal sequence
- The signal sequence is cleaved as the polypeptide chain crosses the membrane, so the amino (N) terminus of the polypeptide chain is exposed in the ER lumen.
- However, translocation of the polypeptide chain across the membrane is halted when the translocon recognizes a transmembrane sequence.
- This allows the protein to exit the translocon laterally and become anchored in the ER membrane.
- Continued translation results in a membrane-spanning protein with its carboxy (C) terminus on the cytosolic side

→ Type II → eg. Transferrin Receptor

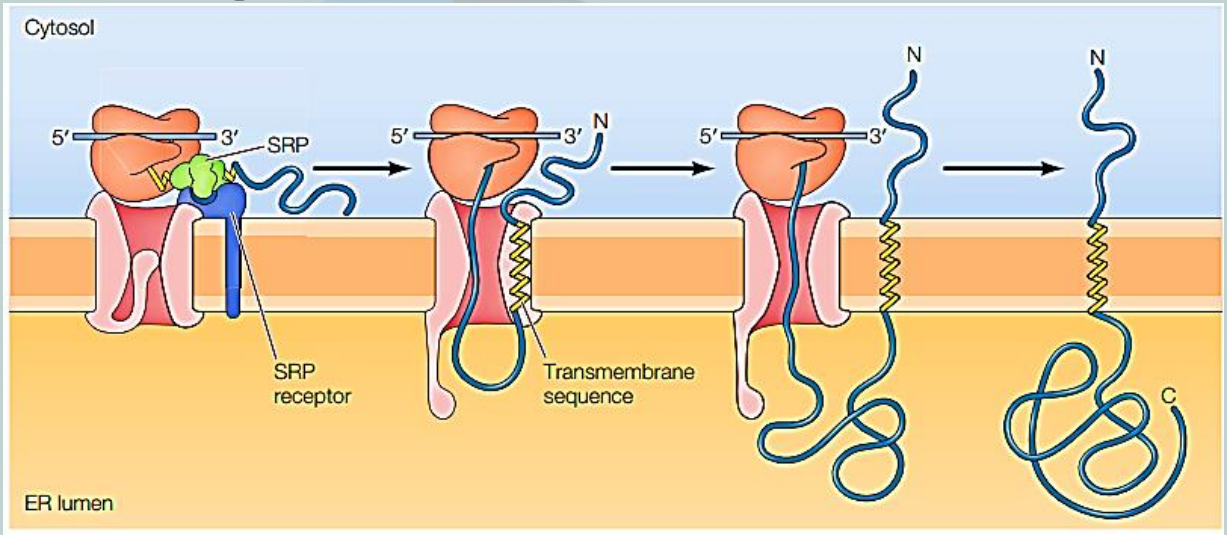
Insertion of membrane proteins via internal transmembrane sequences I

Type - II

N - Ter - Cyto

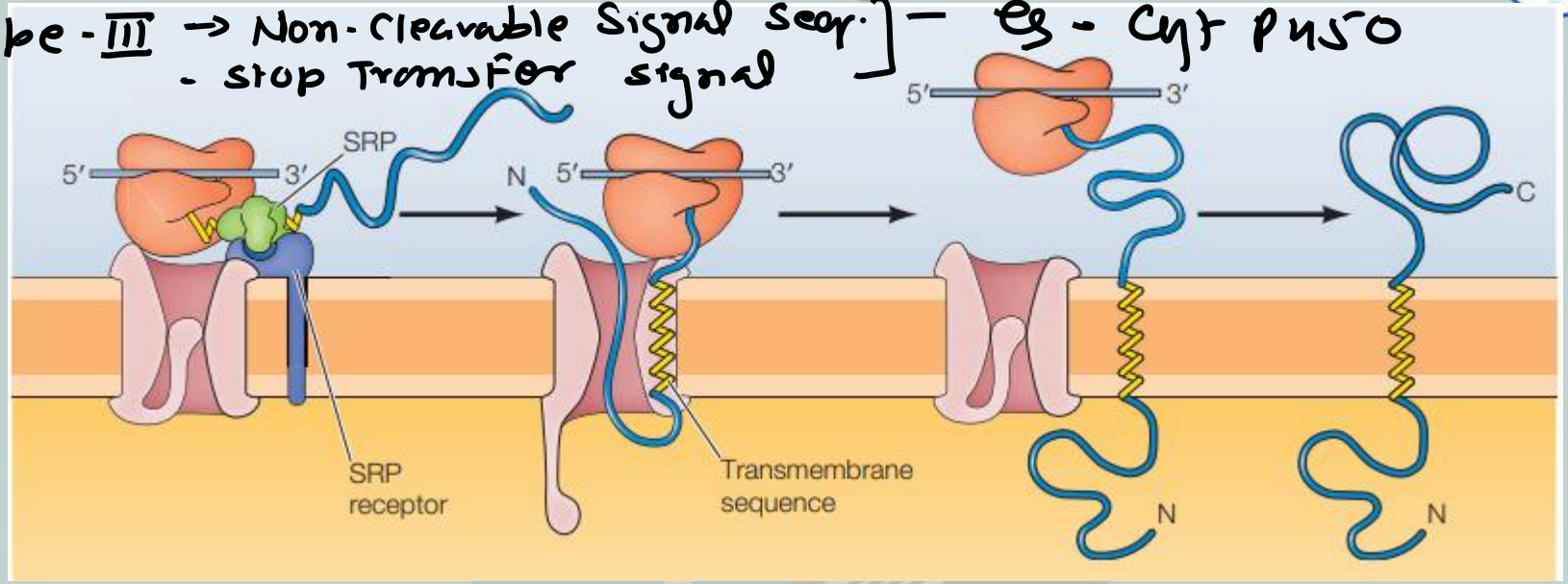
C - Ter - Exo

• Signal peptide
Non Clevable
↓
K/A Start Transfer signal



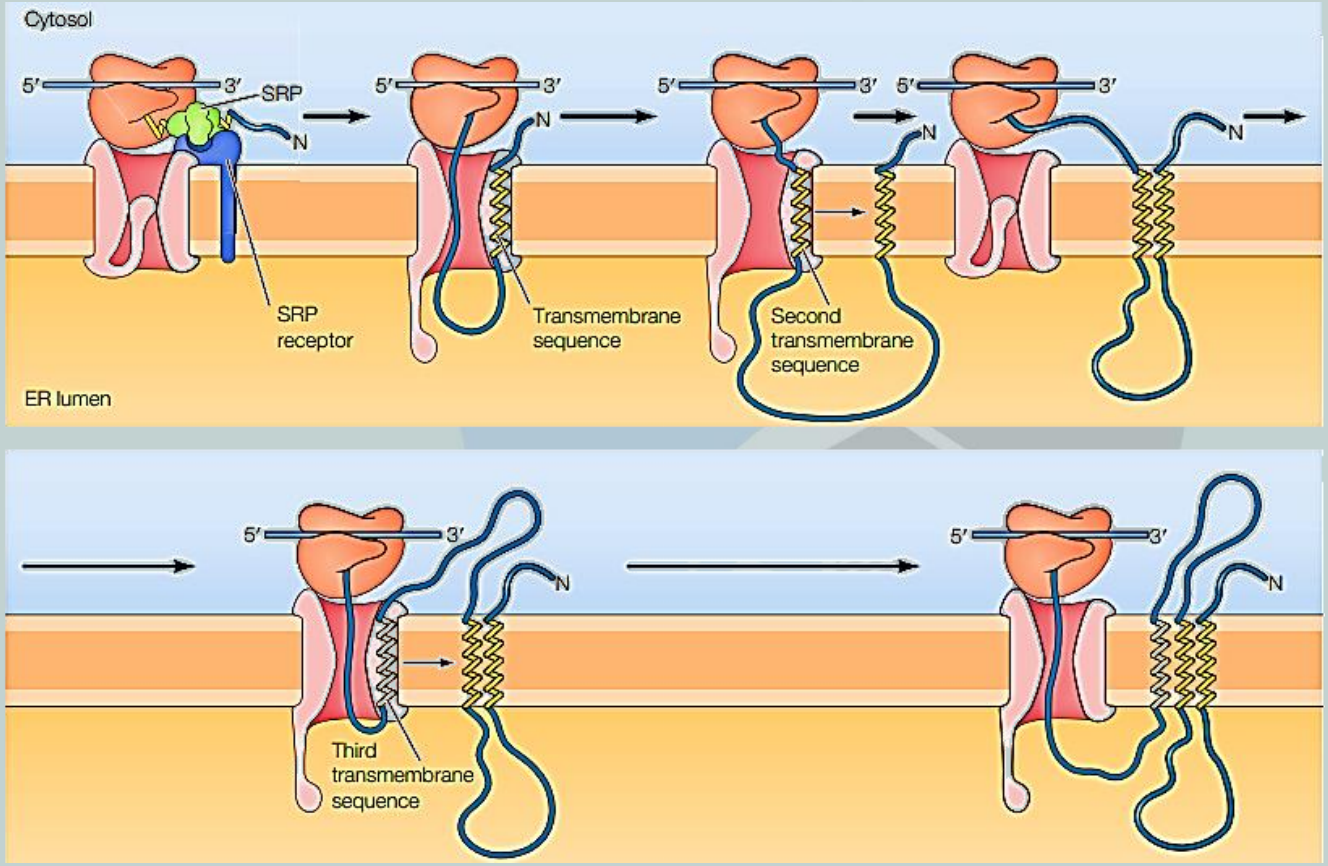
The transmembrane sequence directs insertion of the polypeptide such that its amino (N) terminus is exposed on the cytosolic side. The transmembrane sequence exits the translocon to anchor the protein in the lipid bilayer and the remainder of the polypeptide chain is translocated into the ER as translation proceeds

Type - III → Non-Cleavable Signal seqn.] — eg - Cyt P450
- stop Transfer signal



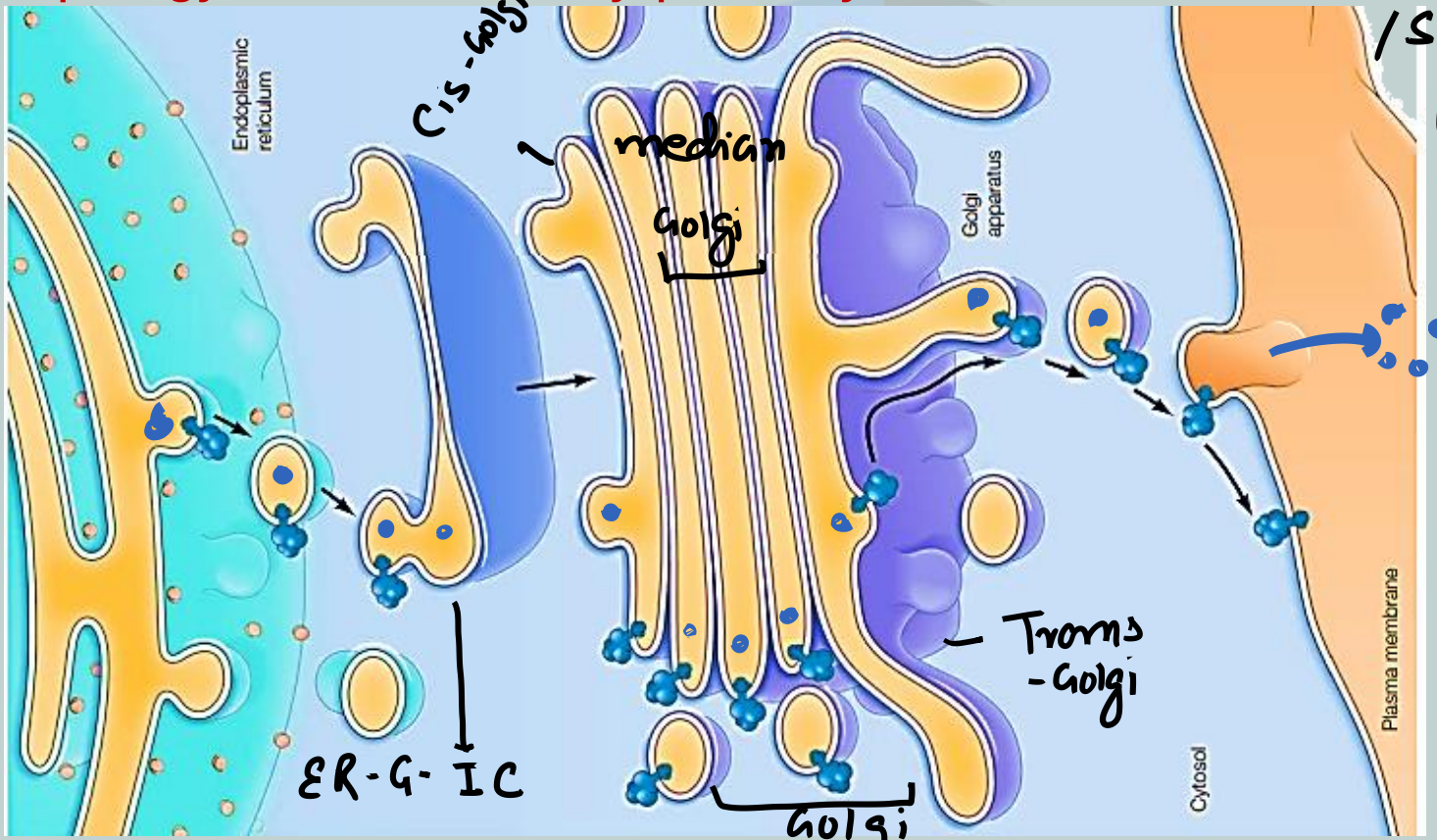
Other internal transmembrane sequences are oriented to direct the transfer of the amino-terminal portion of the polypeptide across the membrane. Continued translation results in a protein that spans the ER membrane with its amino terminus in the lumen and its carboxy (C) terminus in the cytosol.

Insertion of a protein that spans the membrane multiple times [Type IV]

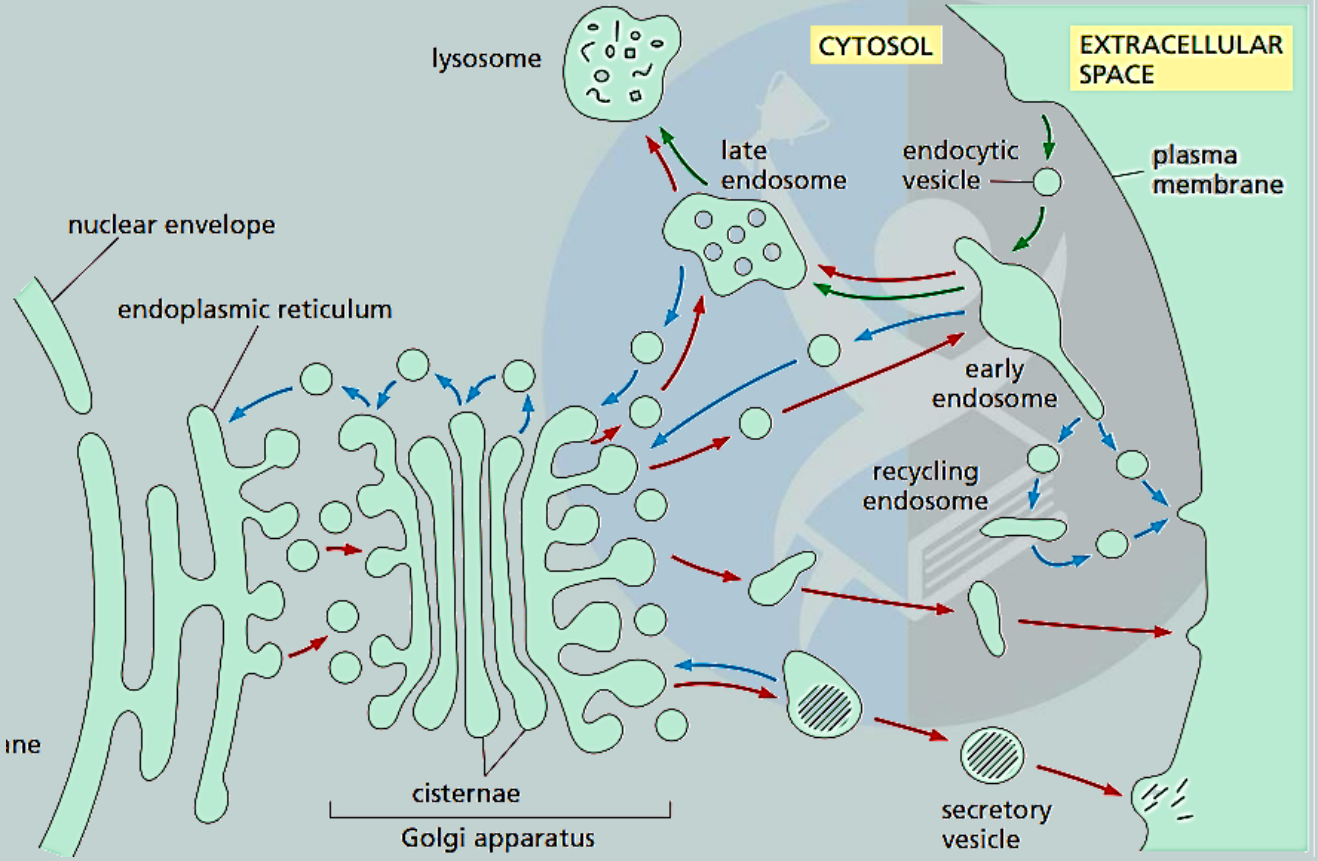


Topology of the secretory pathway

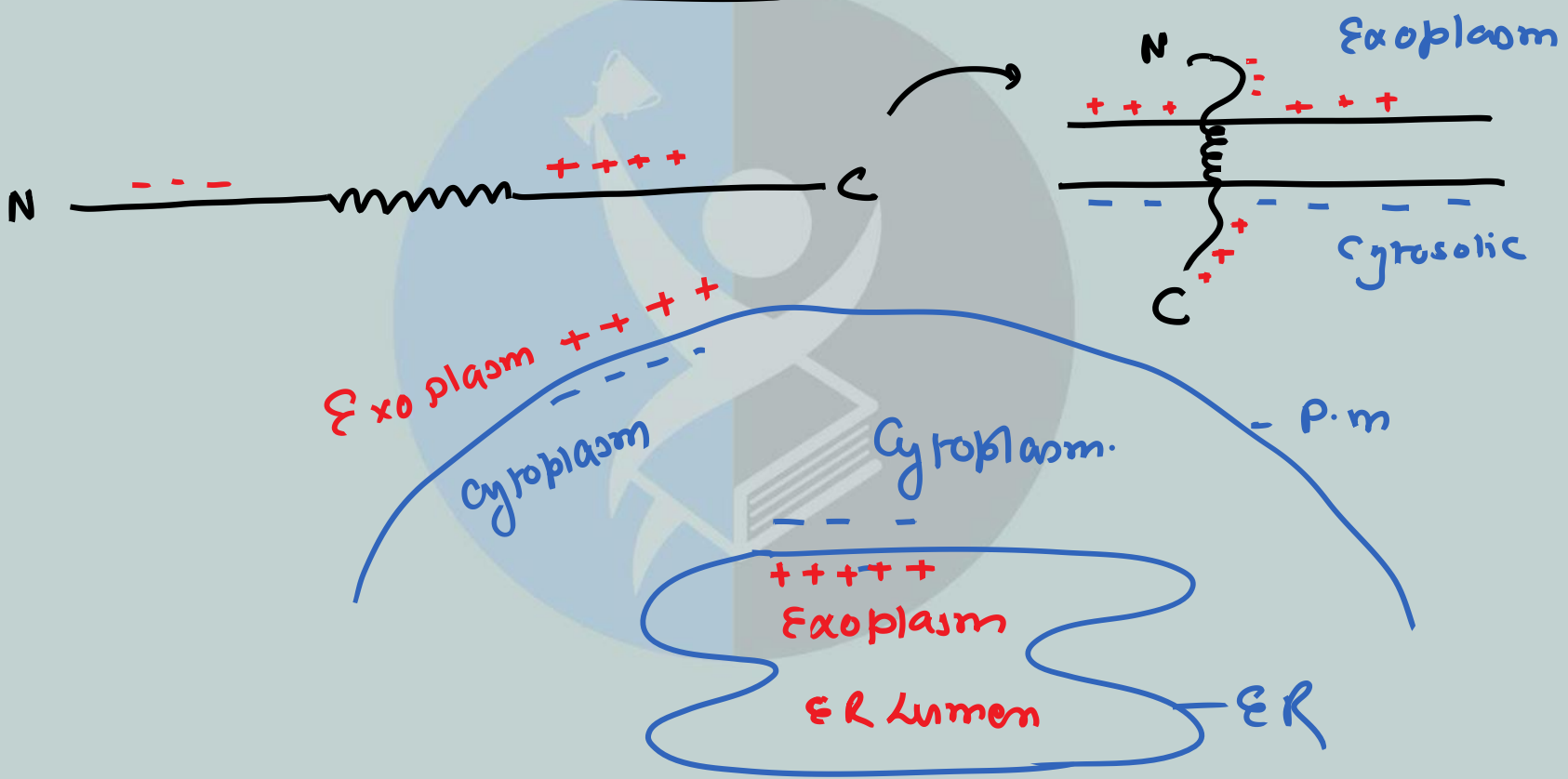
→ membrane protein / Secretory protein

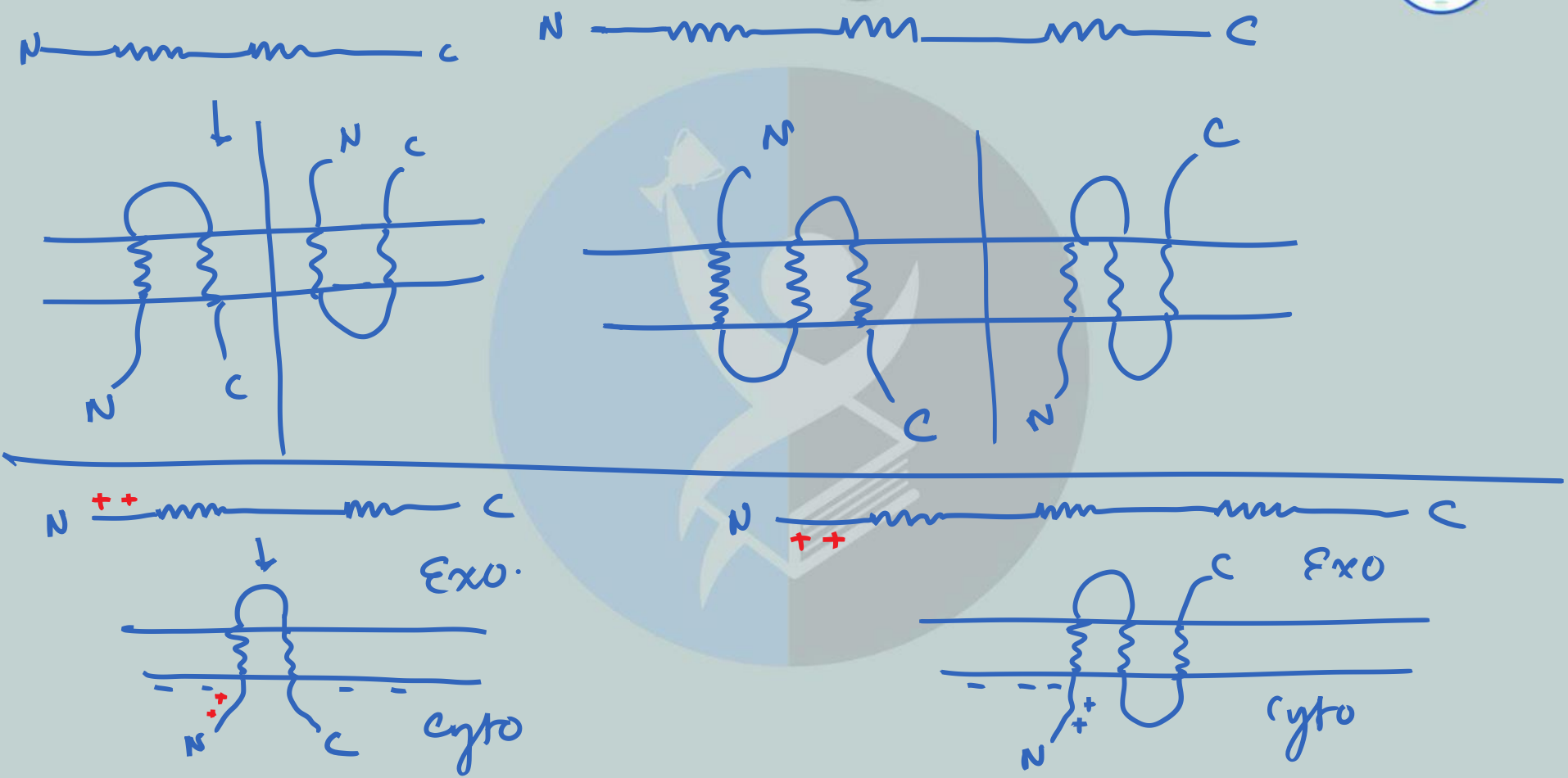


A “road-map” of the secretory and endocytic pathways.



membrane protein - Topology

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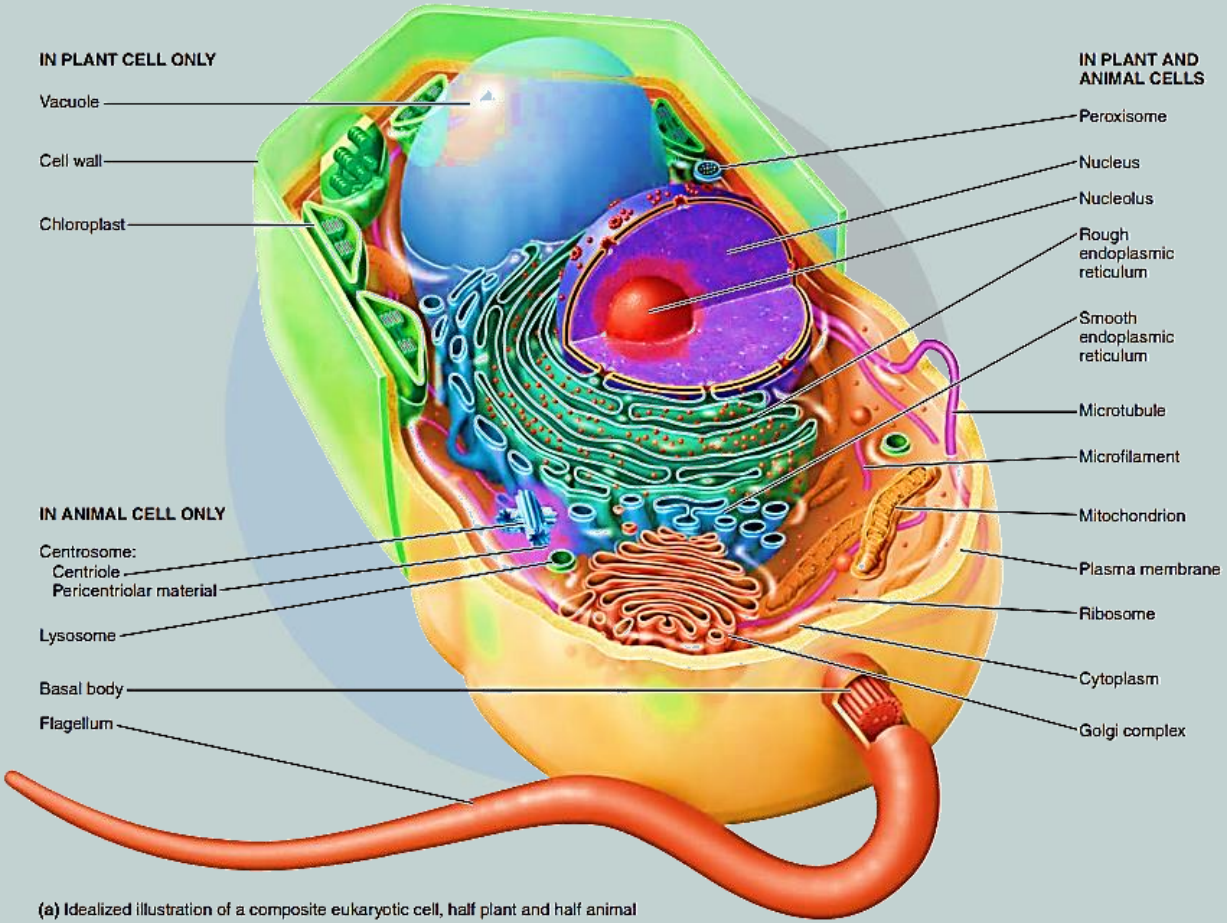
Cell organelles

- RER
- SER

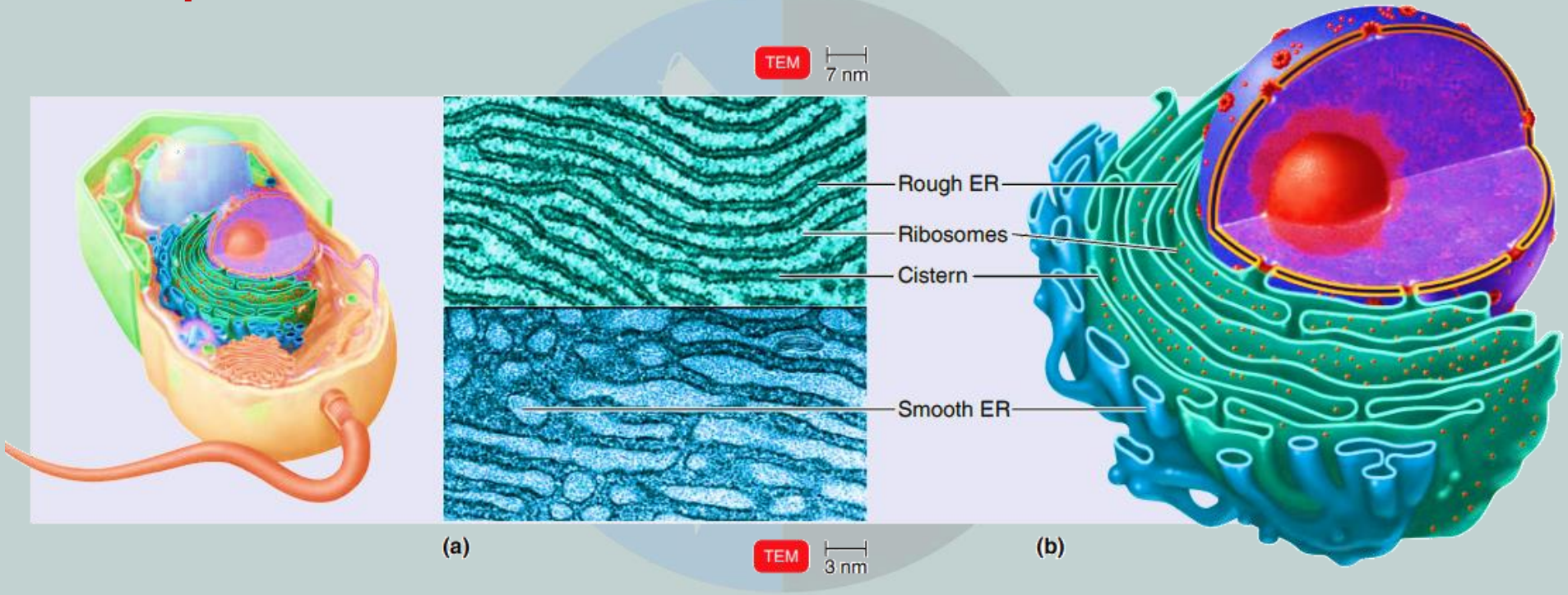
] ER

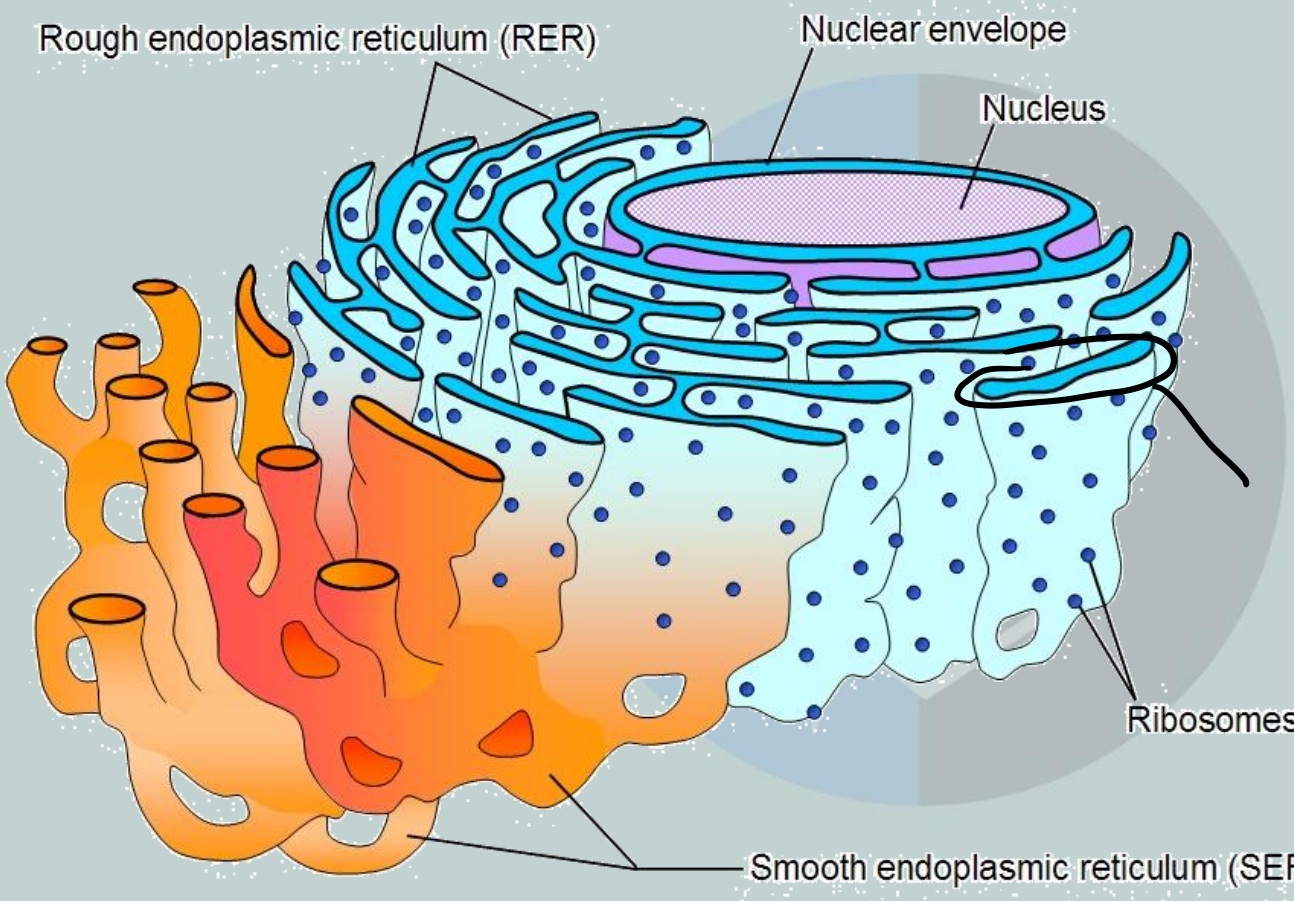
- Golgi
- Lysosome
- Vacuole



(a) Idealized illustration of a composite eukaryotic cell, half plant and half animal

Endoplasmic reticulum




Rough endoplasmic reticulum (RER)
Nuclear envelope
Nucleus
Ribosomes
Smooth endoplasmic reticulum (SER)

SER
RER

Protein synthesis is starts at cytosolic ribosomes

If nascent peptide has N-terminal signal peptide

Translation halts after binding of SRP

Protein is transferred to RER

Secretory protein

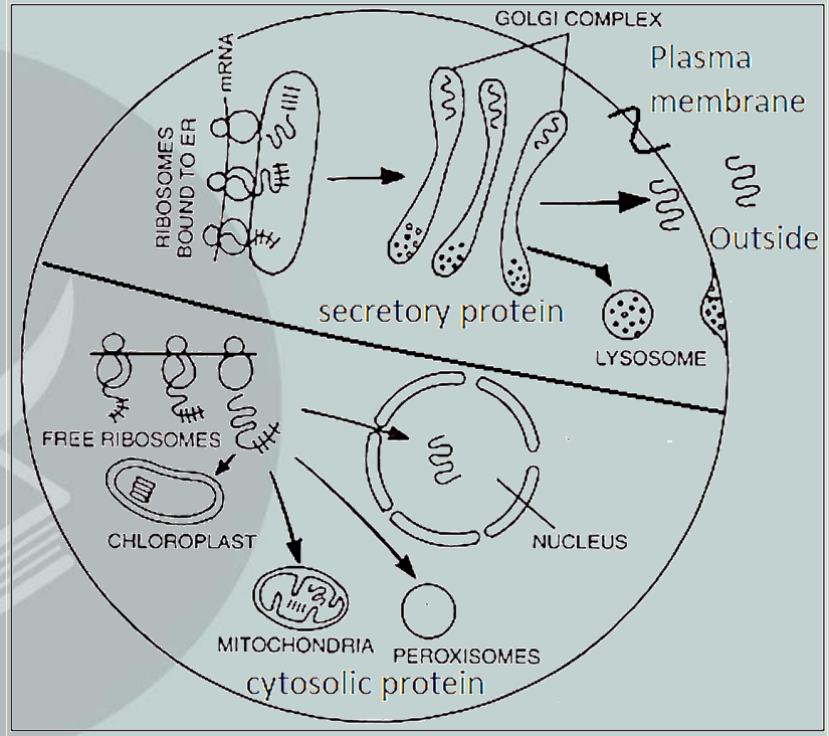
Further can be transported to final destination on basis of signal in peptide

If nascent peptide lack signal peptide

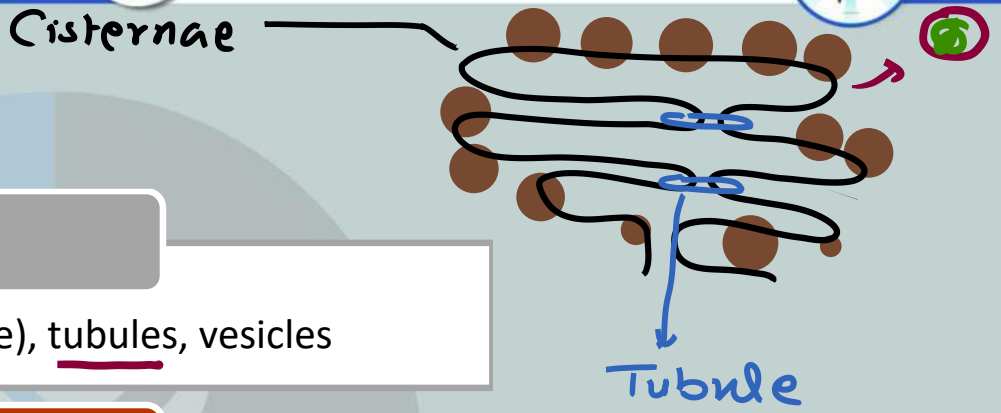
Translation continues in cytosol

Cytosolic protein

Further can be transported to final destination on basis of sorting signal



Rough ER

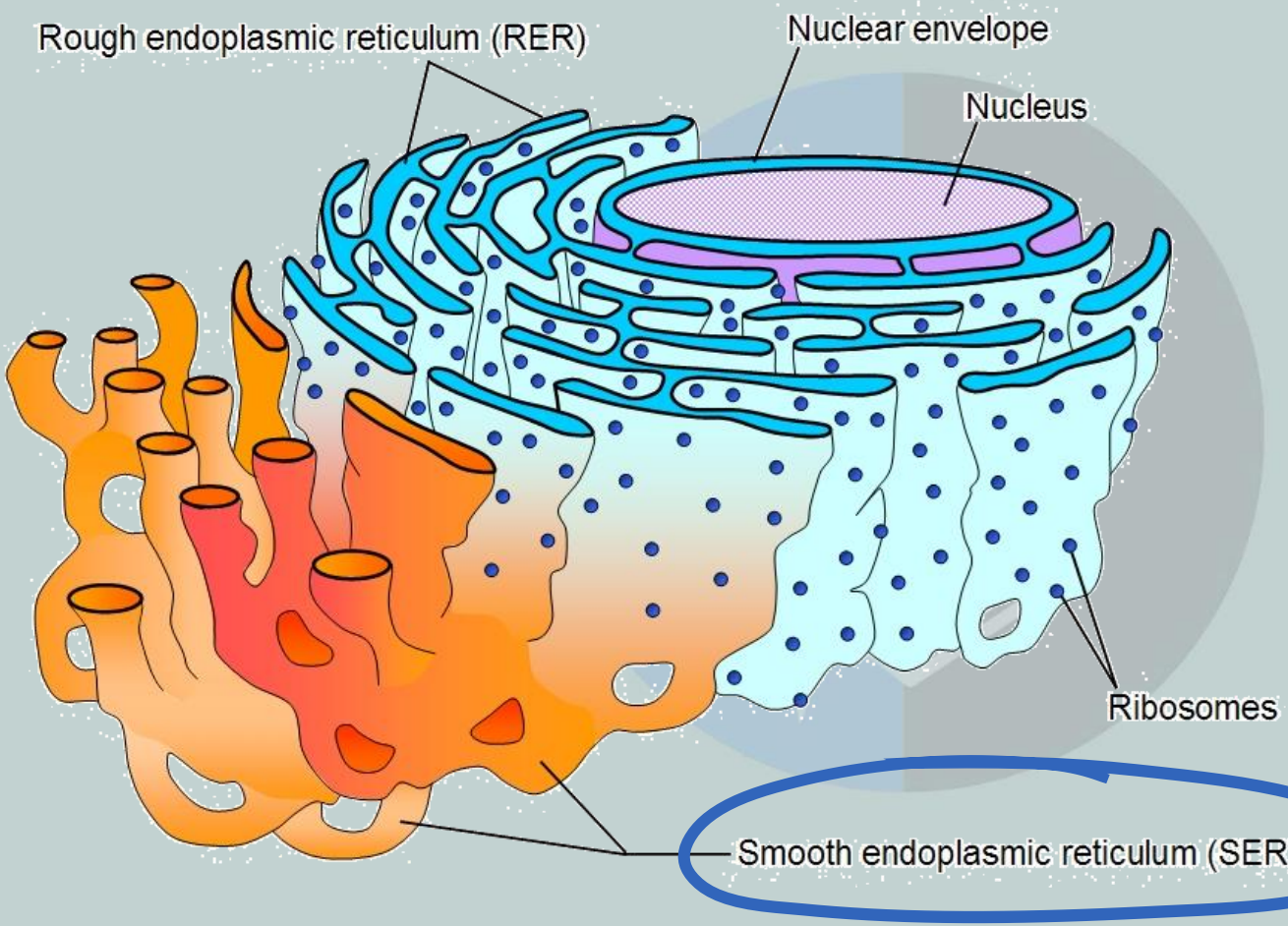


Structure

- Cisternae (well developed with ribosome), tubules, vesicles

Function

- Synthesis of secretory protein ✔
- Protein folding and Quality Control ✔
- Disulphide bond formation ✔
- N-linked glycosylation (occurs on R-chain of Asn of protein) ✔



Tubule well developed

- Site for Synthesis of Phospho-lipid toward Cytosolic Phase

Smooth ER → No Ribosome attached

Structure

- Cisternae, tubules (well developed) and vesicles

Function

PE, PC, PS, PI

- Lipid biosynthesis (Phopholipid, Tri Acyl Glycerol)
- Steriod biosynthesis (Cholesterol, Steroid hormones)
- Detoxification of drugs (Cytochrome P450- Monoxygenase)
- Storage of Ca^{2+} (sarcoplasmic reticulum)
- Glycogen metabolism

① Phopholipid Biosynthesis

- PE, PI, PC, PS → Synthesis occurs toward cytoplasmic Phase of SER

- SER membrane Contain Scrambalase
  ↓
  Symmetrical distribution of SER membrane Lipid

- Golgi & P.m → Contain Flippase & Flappase → maintain Partial asymmetry

- Synthesis of Ceramide occurs on SER membrane

Sphingosine + 1 F.A

- Synthesis of Tri acyl Glycerol

- Glycerolomosomes
  - Far droplet
  - tnt in Adipose tissue

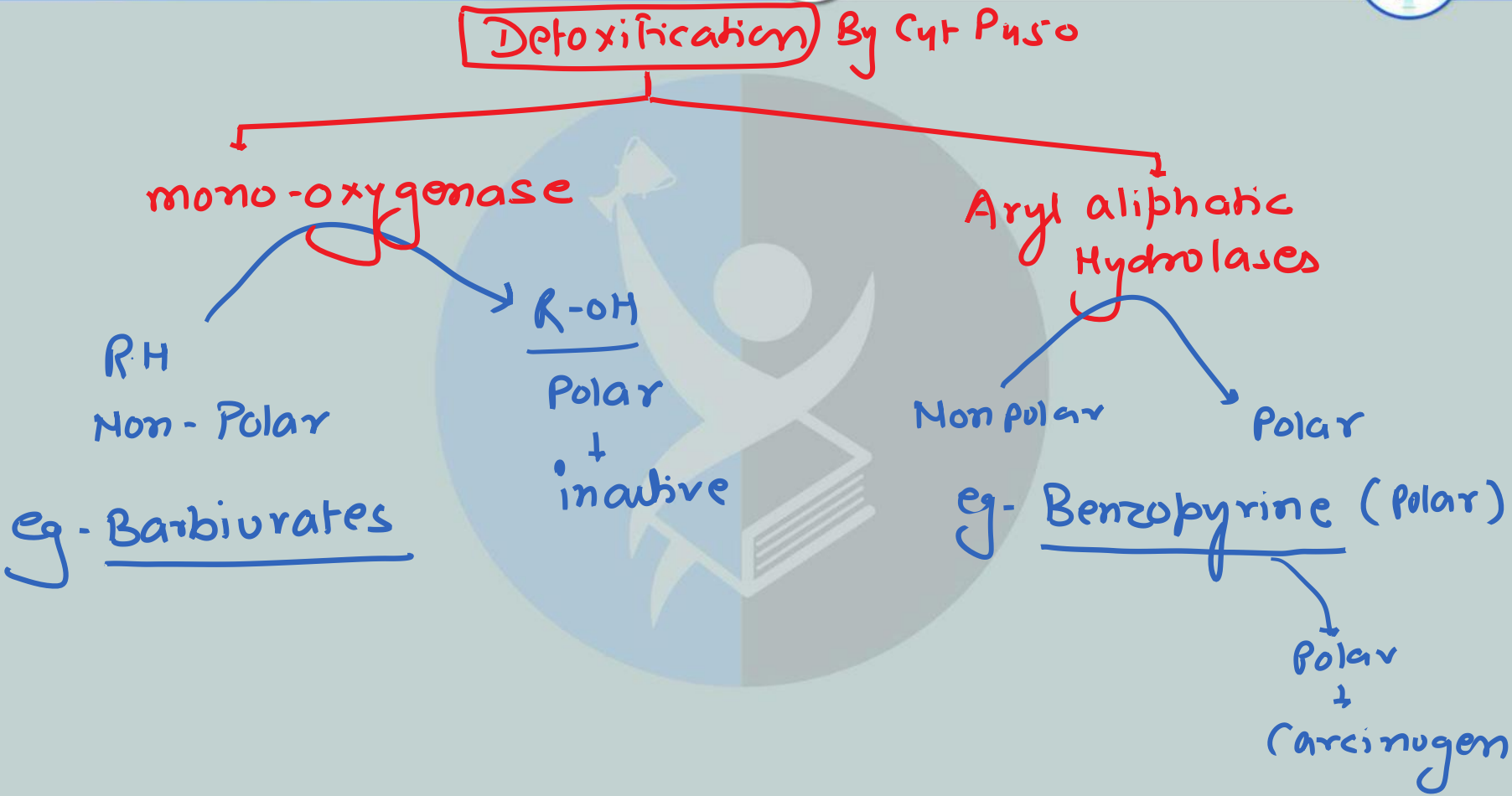
② • Steroid Hormone Synthesis

↳ Precursor - is cholesterol

↳ Estrogen, Progesteron, Aldosteron
Testosteron, Cortisol, Cortison etc.

③ • Detoxification of Drugs

- Cyt-P450 of SER membrane
  ↓
  +nt on SER membrane


Detoxification By Cyt P450
mono-oxygenase
RH
Non-Polar
eg- Barbiurates
R-OH
Polar
↓
inactive
Aryl aliphatic Hydrolases
Non polar
Polar
eg- Benzopyrine (Polar)
Polar
↓
Carcinogen

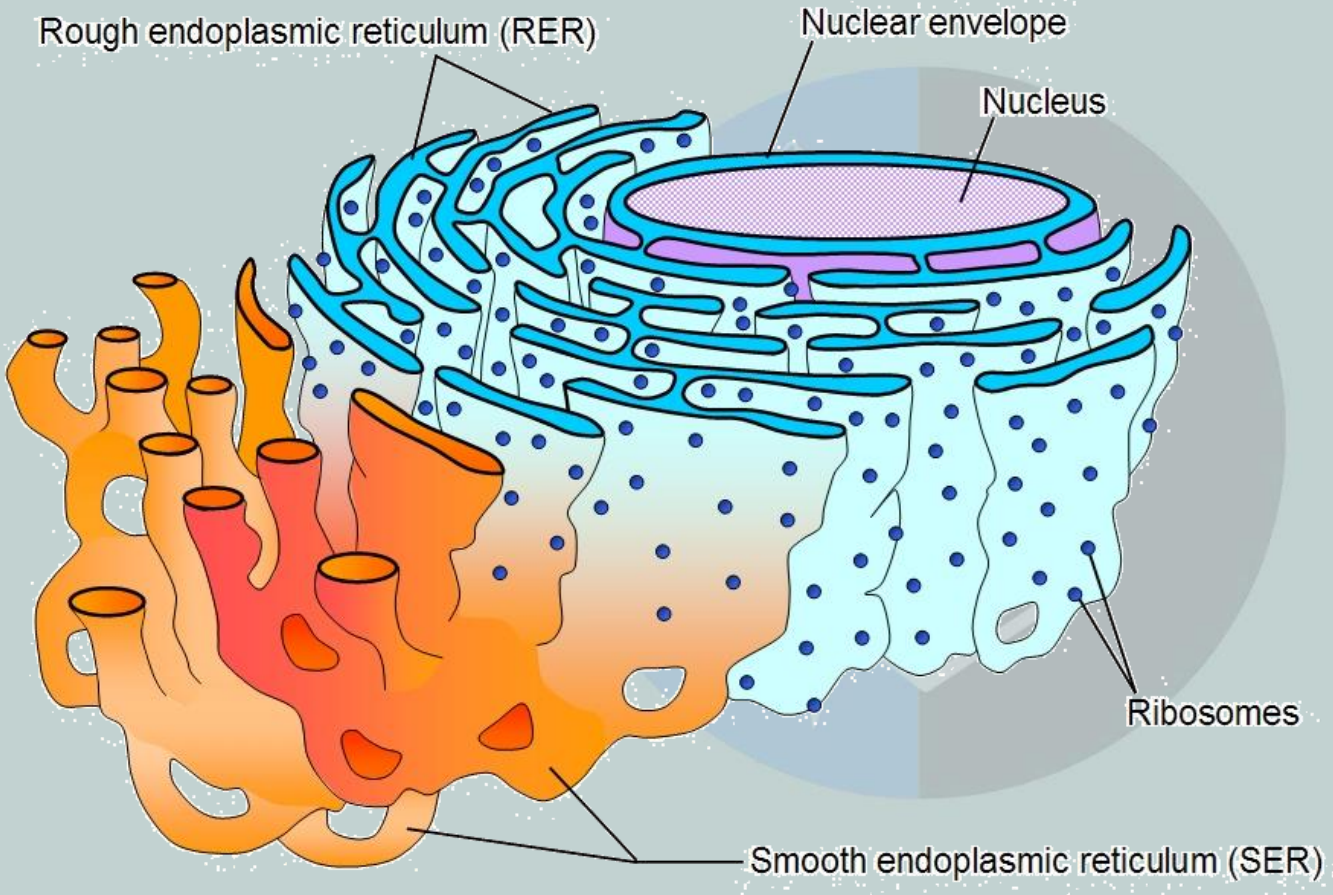
Lysosome

Structure

- Single membrane bound spherical vesicle like structure
- Lumen has acidic pH (4.5) in lumen due to V-type of pump
- Marker membrane protein : LAMP-1
- Have variiety of acid hydrolases

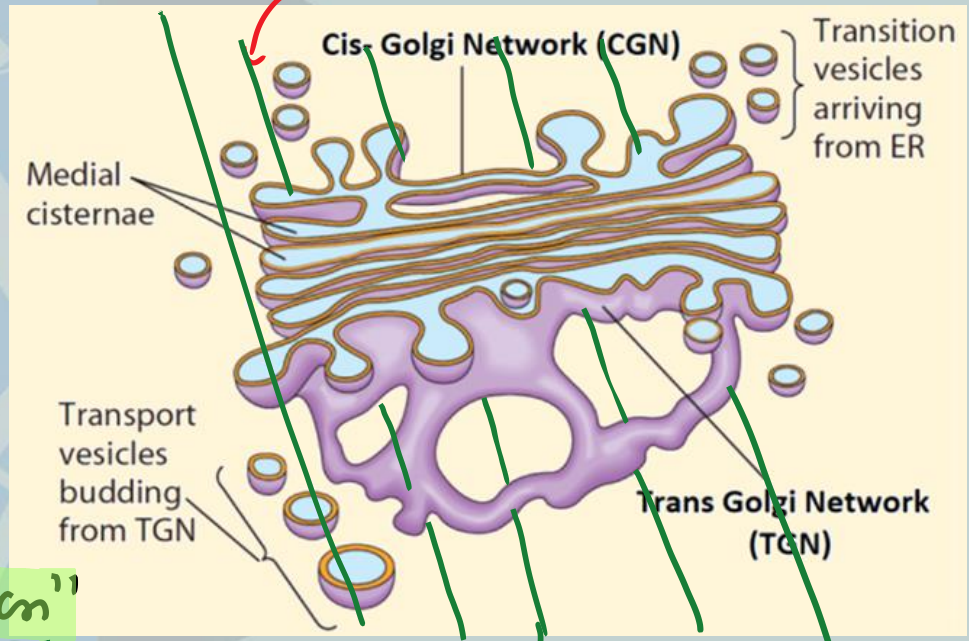
Function

- **Heterophagy:** Digest extra cellular content like bacteria, virus.
- **Autophagy:** Degrade cells own content like non-functional mitochondria. Marker of autophagy is LC3 protein.
- Termed as suicidal bag of cell


Rough endoplasmic reticulum (RER)
Nuclear envelope
Nucleus
Ribosomes
Smooth endoplasmic reticulum (SER)

Golgi Complex

- Golgi complex consist of cisternae, tubules and vesicles.
- It has definite polarity – cis golgi toward RER or nucleus while trans golgi network toward plasma membrane


Cis- Golgi Network (CGN)
Transition vesicles arriving from ER
Medial cisternae
Transport vesicles budding from TGN
Trans Golgi Network (TGN)

RER

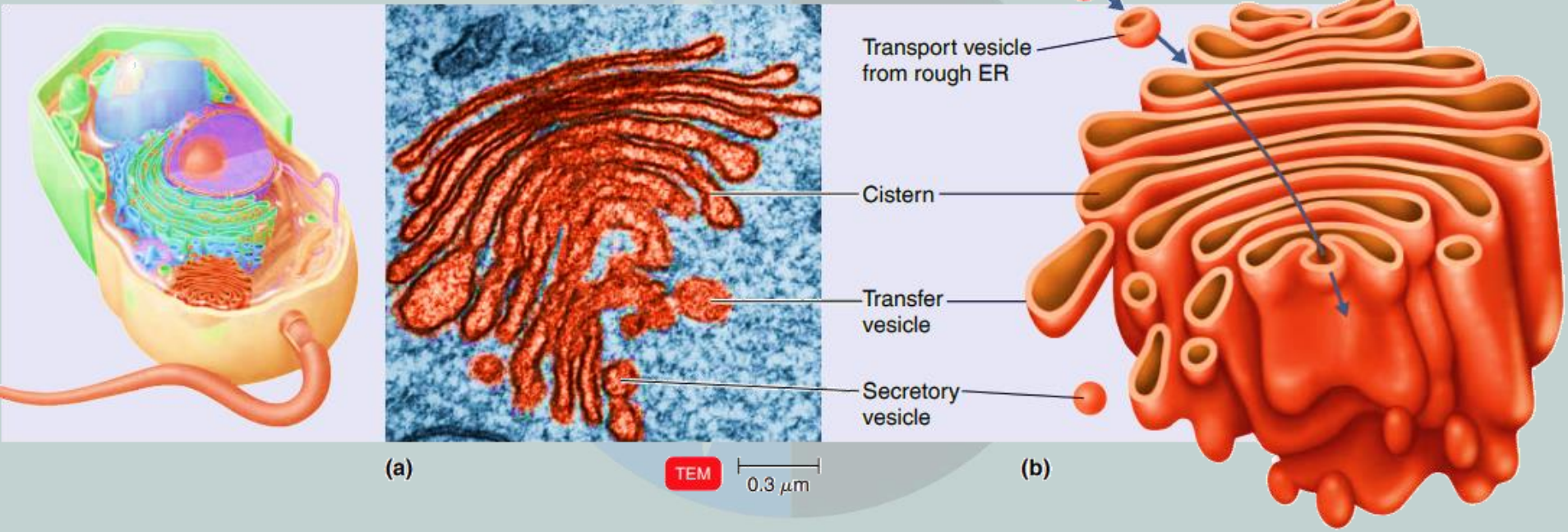
- middle man of cell

→ Present between RER and Plasma membrane

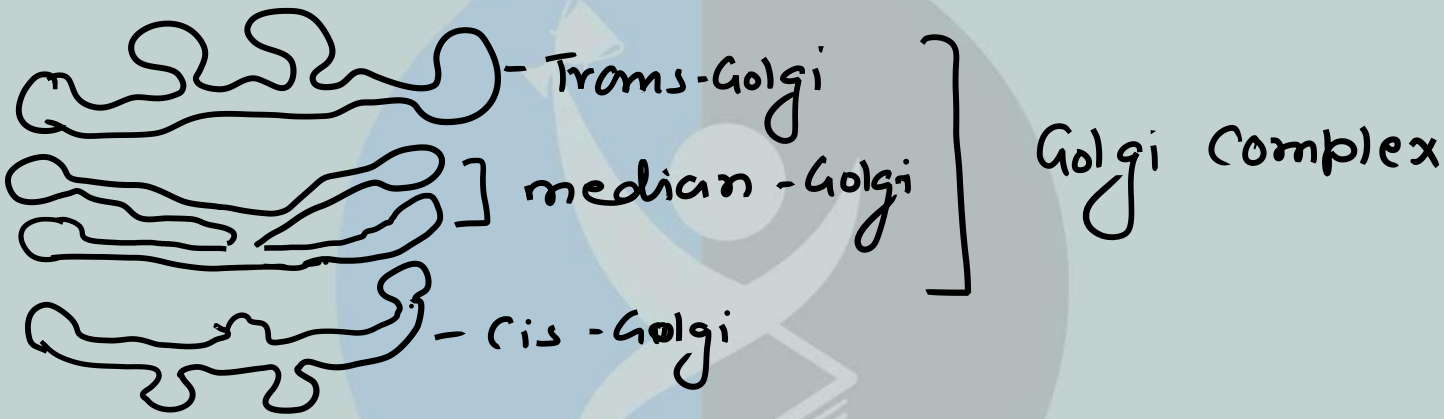
→ Golgi mil in "Zone of Exclusion"

P.M.

Formed By microtubule Network


Transport vesicle from rough ER
Cistern
Transfer vesicle
Secretory vesicle
(a)
TEM
0.3 μm
(b)

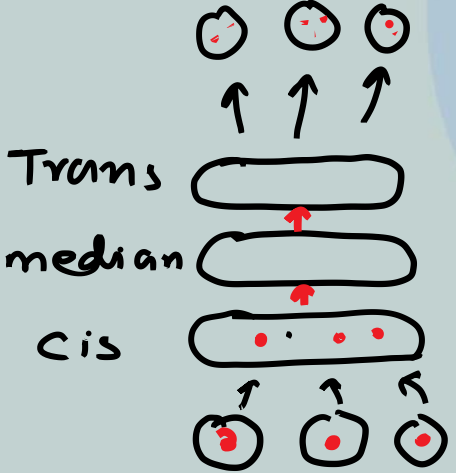
→ microtuble form - Track for vesicular Transport

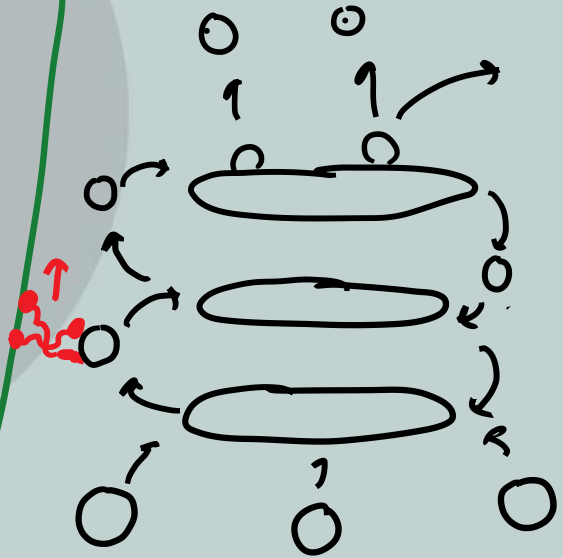


Golgi is Explained by 2 model -

① Cisternal maturation model

② vesicular Transport model ✓





Golgi involve in Protein sorting and targetting

↓

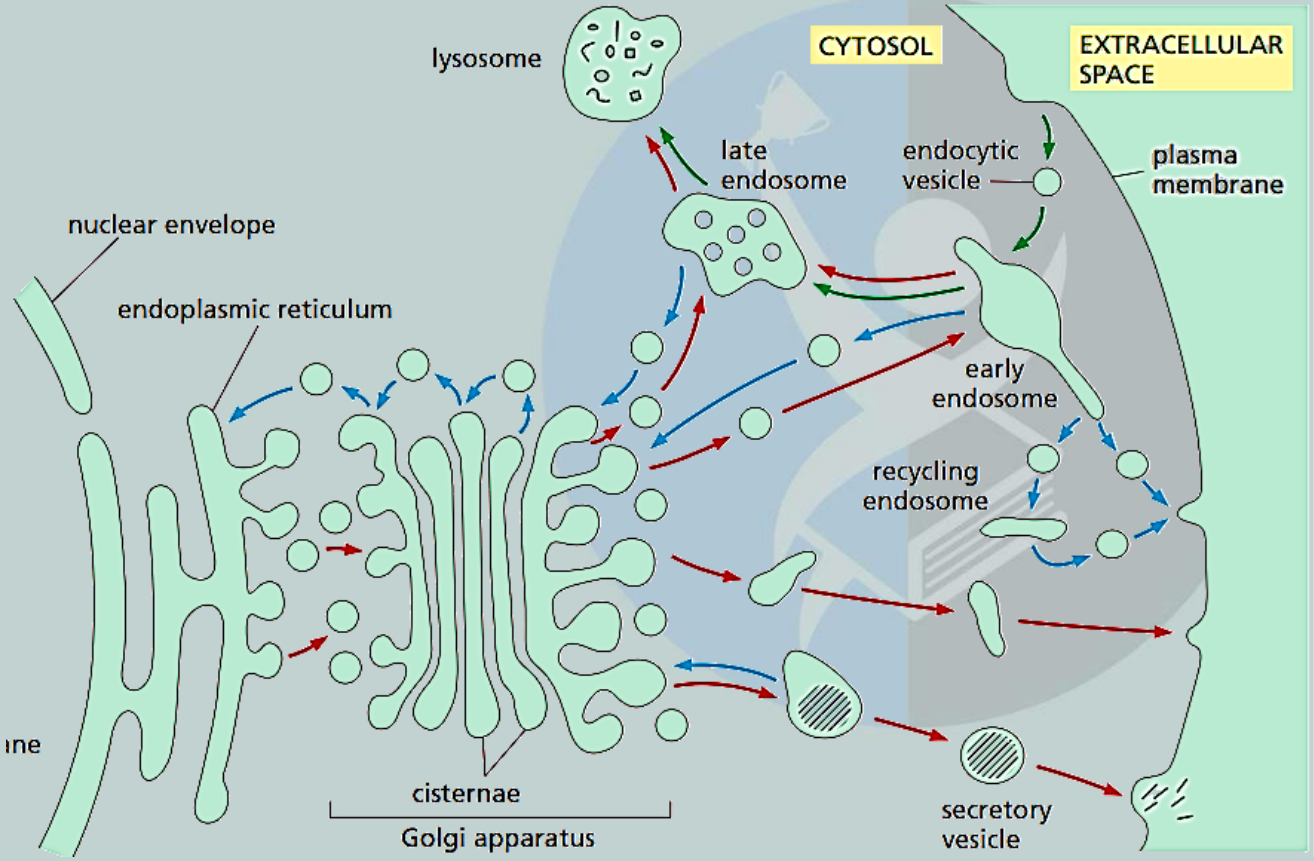
Sorting of Secretory Protein

According to the Signal

↓

Known as Target Signal

A “road-map” of the secretory and endocytic pathways.



→ Secretory Path

From RER → Golgi

↓

Anterograde Path

← Toward RER

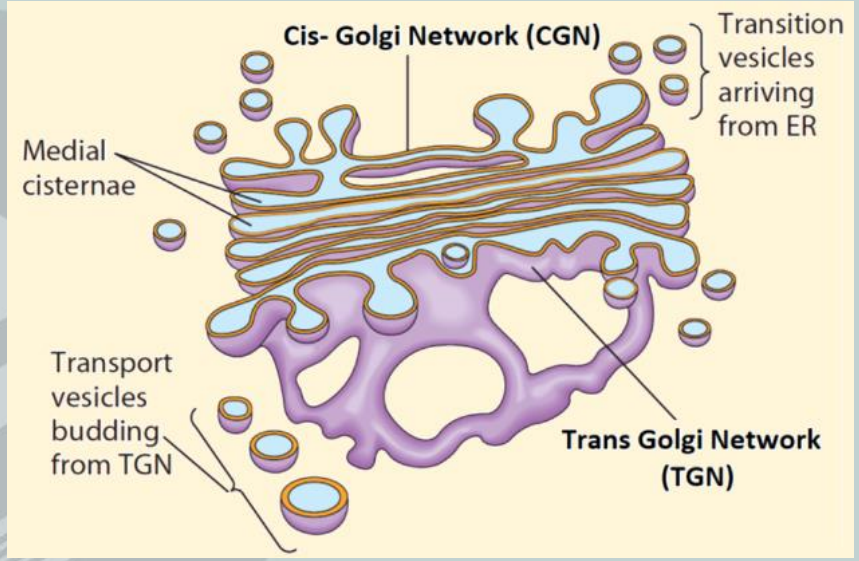
↓

Retrograde Path / Endocytotic Path

Golgi Complex

Important function of Golgi complex are

- Protein sorting and protein secretion
- O-linked glycosylation on R-chain of serine or threonine
- Addition of mannose 6- phosphate for lysosomal proteins
- Carbohydrate biosynthesis – pectin and hemi-cellulose
- Synthesis of sphingolipids
- Finalize N- linked Glycosylation.
- Formation of endosome, lysosome, vacuole.
- Acrosome formation in sperm


Cis- Golgi Network (CGN)
Transition vesicles arriving from ER
Medial cisternae
Transport vesicles budding from TGN
Trans Golgi Network (TGN)

O-linked Glycosylation (Post Translational modification)

↳ Sugar stepwise addition on Ser/Thr → OH group

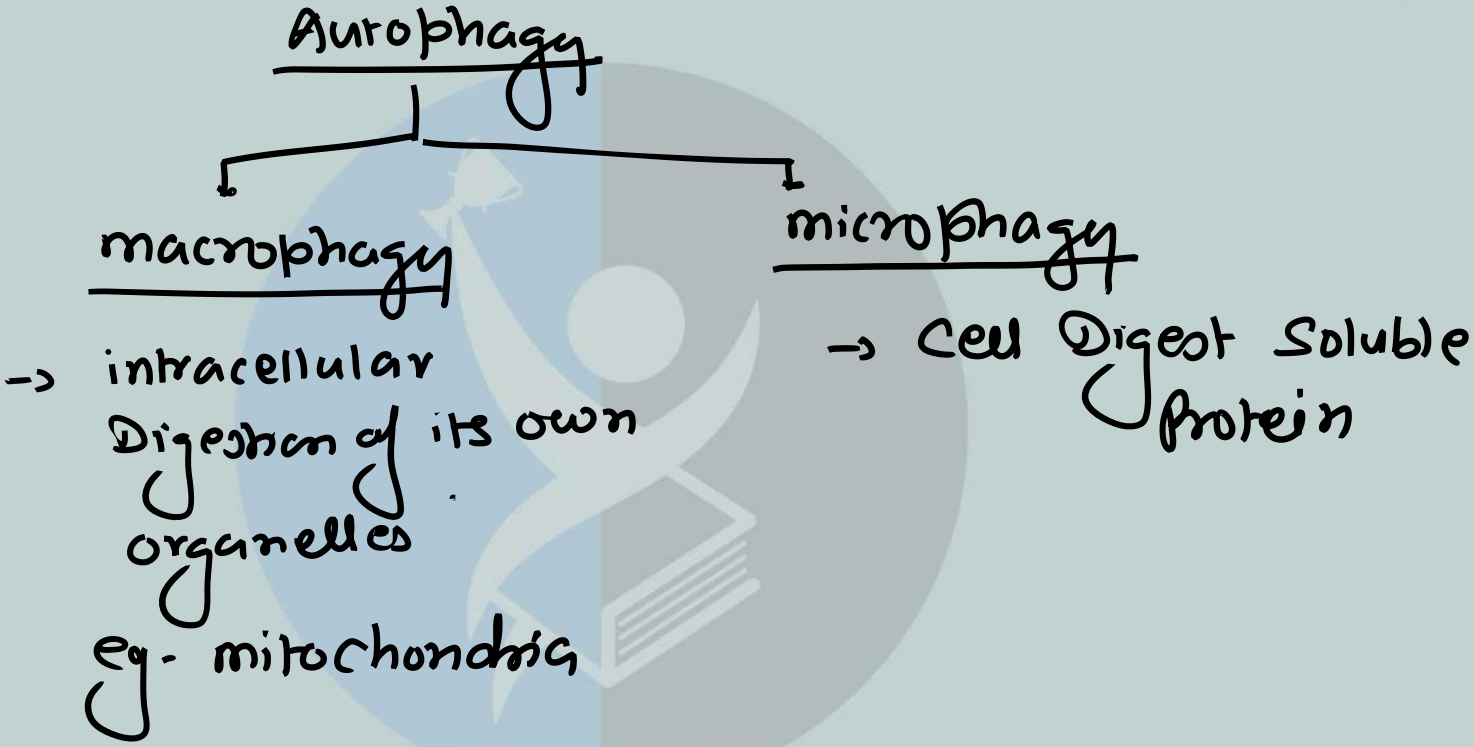
Lysosome

Structure

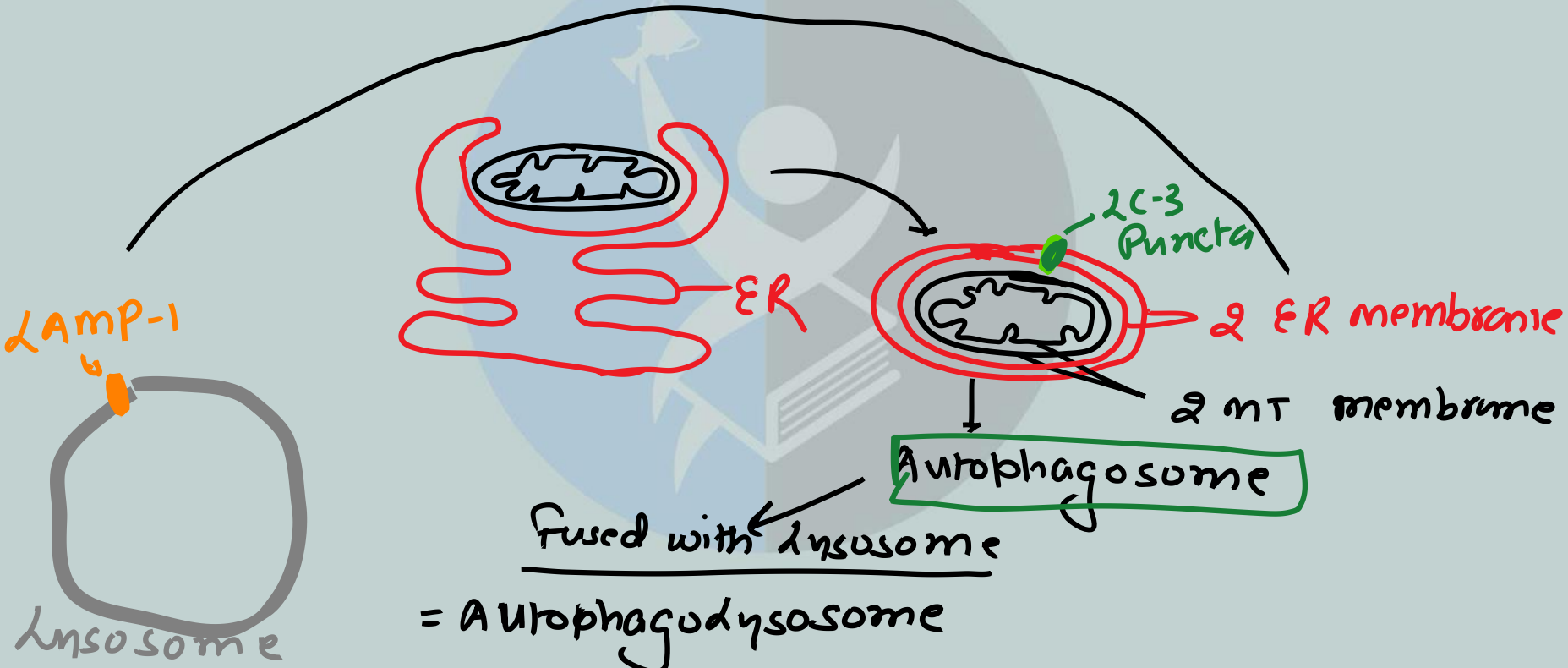
- Single membrane bound spherical vesicle like structure
- Lumen has acidic pH (4.5) in lumen due to V-type of pump [H^+ Pump]
- Marker membrane protein : LAMP-1 [Lysosomal Associated membrane protein-1]
- Have variety of acid hydrolases [Intracellular Digestion]

Function

- **Heterophagy:** Digest extra cellular content like bacteria, virus. → Phagocytosis
- **Autophagy:** Degrade cells own content like non-functional mitochondria. Marker of autophagy is LC3 protein.
- Termed as suicidal bag of cell


Autophagy
macrophagy
→ intracellular Digestion of its own organelles
eg- mitochondria
microphagy
→ Cell Digest Soluble protein

Macrophagy


LAMP-1
Lysosome
ER
LC-3
Puncta
2 ER membrane
2 MT membrane
Autophagosome
Fused with lysosome
= Autophagolysosome

- if LC-3 Puncta is colocalized with LAmp-1

↓

Conformation of Autophagolysosome Formation.

Lysosomal Storage Diseases

Name of Disorder	Mutant Enzyme
Gaucher's disease	Glucocerebrosidase
Tay-Sach's Disease	GMT-2 Gangliosidase or β-N-acetylhexosaminidase,
Pompe II Disease	Alpha-1,4-Glucosidase (glycogen accumulates)
Niemann-Pick Disease	Spingomyelinase
Von-Gerke Disease	Alpha-1,6-Glucosidase (glycogen accumulates)
Hurler Syndrome	α-L-iduronidase (glycosaminoglycans accumulate)
Metachromatic leukodystrophy	Arylsulfatase (cerebroside-sulfatase accumulate)

Vacuole

- Most Prominent Cell organell in plant cell occupy ≈ 70-1. Plant cell volume
- Contain Acid Hydrolases.
- vacuole membrane is called as Tonoplast
  - Contain variety of Transporter

Functions of Vacoule

- It maintains cell turgor and cell shape
- Keeps the chloroplasts closer to light due to push by vacoule.
- Store inorganic ions (like K^+ and Cl^-), salts (such as calcium), and other substances, including toxic byproducts which may interfere with metabolism in cytosol.
- Accumulates toxins which help to protect from herbivores.
- Stores the pigments in flowers and fruits.
- It contains hydrolytic enzymes, help in recycling of cellular constituents especially during senescence.
- Regulates cytosolic pH with help of ATP-dependent V-type of proton pumps

Peroxisomes ~~and Vacuole~~

Similarity with mitochondria

Functions of Peroxisomes

- Oxidation reactions of organic compounds
- Catabolism of very long chain fatty acids, branched chain fatty acids, D-amino acids and polyamines.
- Biosynthesis of plasmalogens and ether phospholipids
- Metabolism of ureides and reactive oxygen species (H_2O_2)
- Beta oxidation of fatty acid and glyoxylate cycle in plants
- Marker enzyme is catalase → H_2O_2 → $H_2O + O_2$
- X-linked adrenoleukodystrophy, Zellweger syndrome (ZS) and Refsum disease are diseases related with peroxisomes.

In Animal

< 16C F.A

↓

- β-oxidation in mitochondria

> 16C F.A

↓

β-oxidation in Peroxysome

- in Plants Compleat β-oxidation occurs in Peroxysome.

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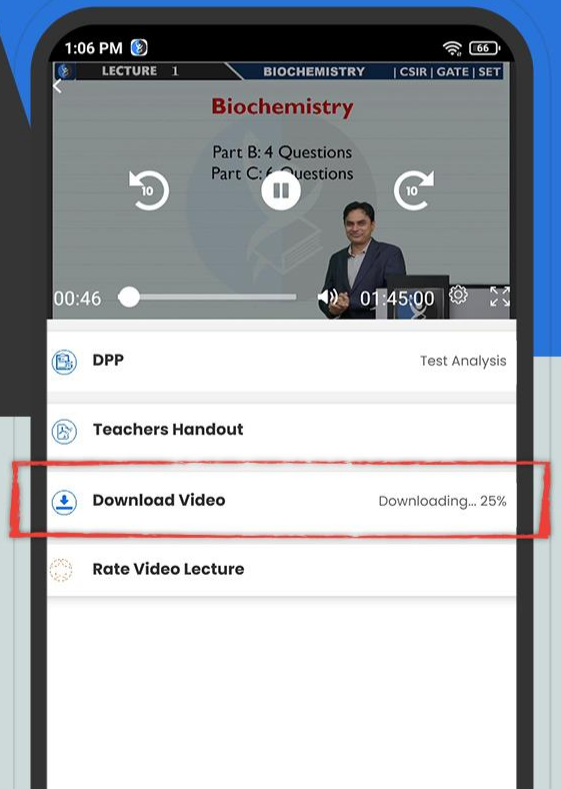

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